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TaqMan® Protein Assays

Sample Prep and Assay Protocol

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TRADEMARKS

Part Number 4449283 Rev. C
02/2011

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About This Guide

Safety information

For general safety information, see this section and Appendix F, "Safety" on page 69.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 70.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

TaqMan® Protein Assays Sample Prep and Assay Protocol

Section 1 Product information

This section covers:

Purpose of the product

The TaqMan® Protein Assays reagents enable detection and relative quantitation of proteins in cultured mammalian cell and tissue lysates.

How to use the TaqMan® Protein Assays reagents

Use the reagents as follows:

- **Sample preparation** – Use one of the kits listed below to prepare cell lysates. These kits are optimized for compatibility with the TaqMan® Protein Assays.
  - Protein Expression Sample Preparation Kit
  - Protein Quant Sample Lysis Kit
- **Assay (relative quantitation experiment)** – Use a TaqMan® Protein Assays kit to detect a protein of interest using proximity ligation technology and real-time PCR.

  Use the TaqMan® Protein Assays kits in conjunction with the TaqMan® Protein Assays Core Reagents Base Kit and the TaqMan® Protein Assays Fast Master Mix.

- **(Optional) Positive control reactions and/or troubleshooting** – Use a Protein Expression Lysate Control Kit in place of user-prepared cell lysates for positive control reactions and for troubleshooting.

For more information

For background information and further details about TaqMan® Protein Assays technology:

- See Appendix D on page 65.
- Refer to the *Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide*. See "Documentation" on page 73.

Kit contents and storage

Sample preparation kits

Kit	Kit part number	Contents	Storage conditions
Protein Expression Sample Preparation Kit	4405443	Cell Lysis Reagent, 2X (3 × 1 mL)	2 to 8°C
		Cell Resuspension Buffer (25 mL)	
Protein Quant Sample Lysis Kit	4448536	Sample Lysis Buffer (25 mL)	2 to 8°C

Control kits

The Protein Expression Lysate Control Kits are for general use. You can use them for positive control reactions and for troubleshooting.

Kit	Kit part number	No. of reactions†	Contents	Storage conditions	
				Long-term	Short-term
Raji Kit; cells express ICAM1 and CTSB	4405448	100	Lysate Control, 500 cells/µL (50 µL)	< –50°C	After initial use, store the **Lysate Dilution Buffer** at 2 to 8°C for up to 3 months.
			Lysate Dilution Buffer (1 mL)	–15 to –25°C‡	
NTERA2 Kit; cells express CSTB, ICAM1, LIN28, NANOG, OCT3/4, and SOX2	4405454	100	Lysate Control, 500 cells/µL (50 µL)	< –50°C	
			Lysate Dilution Buffer (1 mL)	–15 to –25°C‡	

† The number of reactions includes the reactions performed using the 2-fold dilution scheme outlined in this protocol.
‡ If necessary, the Lysate Dilution Buffer can be stored with the Lysate Control.

TaqMan® Protein Assays kits

Kit	Kit part number	No. of reactions	Contents	Storage conditions
Human CSTB Kit	4405465	100	• Assay Probe A, 20X (20 µL) • Assay Probe B, 20X (20 µL) • Assay Probe Dilution Buffer, 1X (0.5 mL)	–15 to –25°C
Human ICAM1 Kit	4405471			
Human LIN28 Kit	4405477			
Human NANOG Kit	4405483			
Human OCT3/4 Kit	4405489			
Human SOX2 Kit	4405495			

Core reagents base kits

Kit	Part number		No. of reactions	Contents	Storage conditions	
	Kit	Top-fill†			Long-term	Short-term
TaqMan® Protein Assays Core Reagents Base Kit (100 rxn)	4405460	4405501	100	DNA Ligase, 500× (10 µL)	–15 to –25°C	After initial use, store the **1× PBS** at 2 to 8°C for up to 3 months.
				Ligase Dilution Buffer, 1× (2 × 1.5 mL)		
				Ligation Reaction Buffer, 20× (0.7 mL)		
				1× PBS, pH 7.4 (1 mL) (Phosphate Buffered Saline)		
				Protease, 100× (10 µL)		
				Universal PCR Assay, 20× (120 µL)		
TaqMan® Protein Assays Core Reagents Base Kit (500 rxn)	4448592	4448591	500	DNA Ligase, 500× (50 µL)	–15 to –25°C	After initial use, store the **1× PBS** at 2 to 8°C for up to 3 months.
				Ligase Dilution Buffer, 1× (15 mL)		
				Ligation Reaction Buffer, 20× (4 mL)		
				1× PBS, pH 7.4 (5 mL) (Phosphate Buffered Saline)		
				Protease, 100× (50 µL)		
				Universal PCR Assay, 20× (600 µL)		

† Top-fill part numbers include both the core reagents base kits and master mix.

Master mix

Kit	Part number		No. of reactions	Contents	Storage conditions
	Kit	Top-fill†			
TaqMan® Protein Assays Fast Master Mix, 2× (100 rxn)	4400088	4405501	100	Fast Master Mix, 2× (1.2 mL)	4°C
TaqMan® Protein Assays Fast Master Mix, 2× (500 rxn)	4448616	4448591	500	Fast Master Mix, 2× (6 mL)	4°C

† Top-fill part numbers include both the core reagents base kits and master mix.

Overall workflow

(Optional) Make your own Assay Probes

Refer to the *TaqMan® Protein Assays Probe Development Protocol*
(See "Documentation" on page 73.)

↓

Prepare the samples (page 15)

Prepare samples using the Protein Expression Sample Preparation Kit (page 19)	**OR**	Prepare samples using the Protein Quant Sample Lysis Kit (page 23)
Prepare the cell lysis solution (page 20)		Prepare the sample lysis solution (page 25)
↓		↓
Count, isolate, and lyse the cells (500 cells/µL) (page 21)		Count (recommended), isolate, and lyse the cells (2500 cells/µL) (page 26)
↓		↓
(Optional) Store the cell lysates (page 22)		(Optional) Determine the total protein concentration (page 26)
↓		↓
		(Optional) Store the cell lysates (page 26)
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Perform the TaqMan® Protein Assays experiment (page 27)

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↓

Perform the binding reaction (page 37)

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Perform the ligation and protease reactions (page 40)

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Perform the real-time PCR (page 43)

↓

Analyze the real-time data (page 47)

Section 2 Prepare the samples

This section covers:

About sample preparation

Kit comparison

To prepare cell lysates that are optimally compatible for use with the TaqMan® Protein Assays, Applied Biosystems recommends one of the following kits:

- Protein Expression Sample Preparation Kit
- Protein Quant Sample Lysis Kit

The differences between the two kits are described in the table below.

	Protein Expression Sample Preparation Kit	Protein Quant Sample Lysis Kit
Sample type	Cultured mammalian cells	
Sample size	Useful for samples in limiting amounts: ~10,000 to 50,000 cells	>50,000 cells
Sample lysis reagent contains carrier protein	Yes	No
Method used to normalize data for TaqMan® Protein Assays analysis	Cell count	Cell count or total protein concentration
Recommended cell lysate concentration	500 cells/µL	2500 cells/µL
Required initial dilution in the TaqMan® Protein Assay	1:2	1:10

Other sample lysis reagents

Other sample lysis reagents are also compatible with the TaqMan® Protein Assays. See Appendix A, "Sample Lysis Reagents" on page 55 for a list of known compatible cell and tissue lysis reagents.

The reagents listed in Appendix A require a minimum of 1:10 lysate dilution in the assay. Appendix A also lists the components in the lysis reagents at concentrations that are known not to interfere with the TaqMan® Protein Assays.

IMPORTANT! Complex biological samples such as cultured cells and tissues express a large assortment of proteins with diverse properties (for example, subcellular localization, hydrophobicity, solubility, modifications, and so on). As a result, it is not generally possible to achieve the best protein assay performance for all proteins in a sample with a single sample preparation or treatment method. It may be necessary to optimize the assay performance for a given target by testing more than one sample lysis reagent.

Before you begin

IMPORTANT! To prepare cell lysates that give consistent and accurate results with TaqMan® Protein Assays, follow the recommendations in this section when you prepare samples with the Protein Expression Sample Preparation Kit or the Protein Quant Sample Lysis Kit.

Best practices for preparing cell lysates

Practice	Reason or details
Prepare cell lysates from mammalian cell lines.	The reagents in the sample preparation kits: • Are optimized for cultured mammalian cells. • Have not been validated with other sample sources.
Perform the cell collection, counting, and lysis steps for one sample at a time, instead of processing several samples together.	Preparing the cell lysate as soon as possible after collecting the cells helps to maximize intact protein in the lysate.
Use ice-cold buffers and solutions to prepare and lyse the cells.	Ice-cold buffer helps to preserve native proteins within the cell before and during lysis.
Prepare fresh cell lysis solution for each experiment.	Adding fresh protease and phosphatase inhibitors to the cell lysis solution helps to protect the released proteins from degradation and maintain their phosphorylation states. • Store the cell lysis solution on ice while you count the cells. • Use the cell lysis solution within 2 hours of preparation.
Obtain accurate cell counts. **Note:** Counting cells is optional, but recommended, for the Protein Quant Sample Lysis Kit.	TaqMan® Protein Assays are normalized to cell input when using the Protein Expression Sample Preparation Kit. TaqMan® Protein Assays can be normalized to cell or protein input when using the Protein Quant Sample Lysis Kit. To obtain accurate cell counts: • Count cells before lysis. • Be sure to count only intact, live cells.
For the Protein Expression Sample Preparation Kit, use the TaqMan® RNase P Detection Reagents to estimate cell equivalents if obtaining cell counts before lysis is not feasible.	Cell count estimates by this method can be used to adjust cell lysates to equivalent cell concentrations for TaqMan® Protein Assays. • All samples should be handled in the same way (for example, all fresh vs. all frozen lysates). • The copy number of the RNase P gene for the cell line of interest must be known.
Minimize freeze-thaw cycles of cell lysates.	If you need to store cell lysates, store the lysates in ~50-µL aliquots at –50 °C or below.
If necessary, adjust the cell lysate concentration of your samples to achieve the optimal protein detection range.	The Protein Expression Sample Preparation Kit is optimized to produce cell lysates at 500 cells/µL. The Protein Quant Sample Lysis Kit is optimized to produce cell lysates at 2500 cells/µL. Depending on the expression level of your protein of interest, you may need to adjust the cell lysate concentration of your samples to optimize detection with the TaqMan® Protein Assays.

Best practices for preparing cell lysates	
Practice	**Reason or details**
Follow good pipetting practices.	Delivery of precise volumes during sample preparation will maximize consistency and accuracy of your results: • Use calibrated pipettes. • Make sure that the pipette tips are well seated, and inspect all tips each time you pipet to confirm uniform liquid transfer. • Avoid creating bubbles when pipetting the reagents.
For preparation of cell lysates *and* real-time PCR with TaqMan® RNase P Detection Reagents, follow good PCR practices.	Because PCR can detect only a few copies of target, it is crucial to avoid contamination of samples that will undergo PCR amplification. • Maintain separate areas and dedicated equipment and supplies for: – Sample preparation – PCR setup – PCR amplification – Analysis of PCR products • Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890). • Use a positive-displacement pipette or aerosol-resistant pipette tips. • Follow proper dispensing techniques to prevent aerosols. • Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation). • Change gloves whenever you suspect that they are contaminated. • Never bring amplified PCR products into the PCR setup area. • Centrifuge sample tubes before opening. Open and close sample tubes carefully. Try not to splash or spray PCR samples. • Keep reactions and components capped as much as possible.

Prepare samples using the Protein Expression Sample Preparation Kit

The Protein Expression Sample Preparation Kit procedure is a single-tube lysis procedure that is designed to generate a cell lysate at ~500 cell equivalents/µL. This lysate is then diluted during TaqMan® Protein Assays setup.

Note: If you are using the Protein Quant Sample Lysis Kit, see page 23 for sample preparation procedures.

User-supplied materials

The materials listed in this section are required for sample preparation, but are not included in the Protein Expression Sample Preparation Kit. Unless otherwise indicated, all items are available from major laboratory suppliers (MLS).

Sample

The Protein Expression Sample Preparation Kit reagents and procedure have been optimized for preparing cell lysates from cultured mammalian cells. Follow your laboratory's procedures for culturing mammalian cells.

Reagents

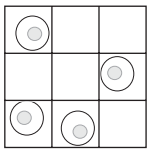
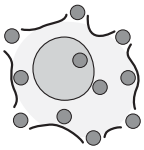
Reagent	Source†
Calbiochem® Protease Inhibitor Cocktail Set I	EMD Chemicals Inc. (Calbiochem PN 539131)
Calbiochem® Phosphatase Inhibitor Cocktail Set II	EMD Chemicals Inc. (Calbiochem PN 524625)
Molecular biology grade nuclease-free water	MLS
1× Phosphate-buffered saline (PBS), pH 7.4	MLS

† For the SDS (Safety Data Sheet) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

General laboratory equipment

Item	Source
Disposable gloves	MLS
Pipette tips (aerosol-resistant)	MLS
Pipettes (positive/air-displacement or multichannel)	MLS
Polypropylene tubes (various sizes)	MLS
Vortexer	MLS
Centrifuge	MLS
One of the following: • Hemocytometer • Countess® Automated Cell Counter	 • MLS • Invitrogen (PN C10227)

Workflow: Prepare samples using the Protein Expression Sample Preparation Kit

Prepare the cell lysis solution (page 20) ↓	
Count, isolate, and lyse the cells (500 cells/µL) (page 21) ↓	 
(Optional) Store the cell lysates (page 22)	

About cell sample concentration

TaqMan® Protein Assays relative quantitation experiments are highly dependent on the accurate quantitation of cells in the test samples.

When using the Protein Expression Sample Preparation Kit to prepare lysates for the TaqMan® Protein Assays kits, Applied Biosystems recommends that you prepare 500 cells/µL per sample. This will be diluted to an initial concentration of 250 cells/µL during assay setup. However, you may need to use a higher cell sample concentration to detect low-abundance proteins. Do not prepare lysates with more than 1000 cells/µL, as excess cellular material may generate false-positive signals in the TaqMan® Protein Assay.

Prepare the cell lysis solution

IMPORTANT! Prepare the cell lysis solution immediately before you lyse the cells. If you are preparing more than one cell lysate sample, you can prepare sufficient cell lysis solution for all of your samples, but you must use the cell lysis solution within 2 hours. Discard any unused solution after 2 hours.

1. Thaw or place the following reagents on ice:
   - Cell Lysis Reagent
   - Protease inhibitor, such as the Calbiochem® Protease Inhibitor Cocktail Set I
   - Phosphatase inhibitor, such as the Calbiochem® Phosphatase Inhibitor Cocktail Set II

Note: The Calbiochem Protease Inhibitor Cocktail Set I and the Calbiochem Phosphatase Inhibitor Cocktail Set II are not included in the Protein Expression Sample Preparation Kit. The Calbiochem Phosphatase Inhibitor Cocktail Set II is supplied as a 100× solution in water.

2. If necessary, reconstitute the Calbiochem Protease Inhibitor Cocktail Set I to a 100× stock solution:
   a. Add 1 mL of molecular biology grade nuclease-free water to the vial containing the Calbiochem Protease Inhibitor Cocktail Set I (lyophilized powder).
   b. Briefly vortex the reconstituted Calbiochem Protease Inhibitor Cocktail Set I.
   c. Place the vial on ice.

      Note: You can store the reconstituted Protease Inhibitor Cocktail solution at –20°C for repeated use.

3. Combine the components listed below.

Component	Volume (µL)†
Cell Lysis Reagent, 2×	500
Calbiochem® Phosphatase Inhibitor Cocktail Set II, 100×	5
Calbiochem® Protease Inhibitor Cocktail Set I, 100×	5
Total volume of cell lysis solution	**510**

† These volumes are sufficient for preparing up to 1 mL of cell lysate. When preparing lysates at the recommended concentration of 500 cells/uL, 0.5 mL of the 2× cell lysis solution is sufficient to lyse 500,000 cells.

4. Mix gently, then briefly centrifuge the tube.
5. Place the cell lysis solution on ice.

IMPORTANT! After preparing the cell lysis solution, proceed *immediately* to "Count, isolate, and lyse the cells" below.

Count, isolate, and lyse the cells

For best results, perform the steps below for one sample at a time, instead of performing the steps for several samples at once.

1. Collect the sample cells according to your laboratory protocol.
2. Count the cells using, for example, a hemocytometer or the Countess® Automated Cell Counter. (See Appendix B, "Obtaining Cell Counts" on page 57.)
3. Place the following reagents on ice:
   - Cell Resuspension Buffer
   - Cell lysis solution (from page 20)
   - 1× PBS, pH 7.4

4. Centrifuge the cells at 500 × g for 4 minutes.
5. Wash the cells twice with ice-cold 1× PBS (to remove residual media).
6. Centrifuge the cells again at 500 × g for 4 minutes.
7. Remove all of the supernatant, being careful not to disturb the cell pellet, then place the tube on ice.
8. Resuspend the cell pellet by adding sufficient ice-cold Cell Resuspension Buffer to achieve a concentration of 1000 cells/µL.

   If the total volume is >500 µL, transfer an aliquot (for example, 500 µL) of the resuspended cells to a fresh tube, then use the fresh tube of cells for steps 9 and 10.
9. Add a volume of ice-cold cell lysis solution equal to the volume in the tube to achieve a concentration of 500 cells/µL (for example, 500 µL).

   As an example, adding 100 µL of cell lysis solution to a 100-µL suspension of cells at 1000 cells/µL would generate 200 µL of lysate at 500 cells/µL.
10. Mix the solution thoroughly by gently pipetting up and down several times (avoiding excess detergent foaming), then place the cell lysate on ice.

(Optional) Store the cell lysates

Applied Biosystems recommends that you perform the TaqMan® Protein Assays experiment soon after you prepare the cell lysates. If you do not plan on performing the TaqMan® Protein Assays experiment within a few hours after preparing the cell lysates, Applied Biosystems strongly recommends that you store the cell lysates as follows:

1. Divide the prepared cell lysates into aliquots of ~50 µL.
2. Quick-freeze by placing the cell lysate tubes in a dry-ice/ethanol bath.
3. Store the cell lysates at –50°C or below (for example, –70°C).

Prepare samples using the Protein Quant Sample Lysis Kit

The Protein Quant Sample Lysis Kit procedure is a single-tube lysis procedure that is designed to generate a cell lysate at ~2500 cell equivalents/µL. This lysate is then diluted during TaqMan® Protein Assays setup.

Note: If you are using the Protein Expression Sample Preparation Kit, see page 19 for sample preparation procedures.

User-supplied materials

The materials listed in this section are required for sample preparation, but are not included in the Protein Quant Sample Lysis Kit. Unless otherwise indicated, all items are available from major laboratory suppliers (MLS).

Sample

The Protein Quant Sample Lysis Kit reagents and procedure have been optimized for preparing cell lysates from cultured mammalian cells or tissues. Follow your laboratory's procedures for culturing mammalian cells or tissues.

Reagents

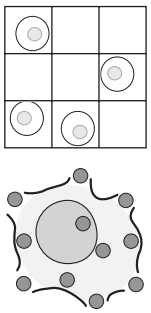
Reagent	Source†
Calbiochem® Protease Inhibitor Cocktail Set I	EMD Chemicals Inc. (Calbiochem PN 539131)
Calbiochem® Phosphatase Inhibitor Cocktail Set II	EMD Chemicals Inc. (Calbiochem PN 524625)
Molecular biology grade nuclease-free water	MLS
1X Phosphate-buffered saline (PBS), pH 7.4	MLS
(Optional) Micro BCA™ Protein Assay Kit	Pierce (PN 23235)

† For the SDS (Safety Data Sheet) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

General laboratory equipment

Item	Source
Disposable gloves	MLS
Pipette tips (aerosol-resistant)	MLS
Pipettes (positive/air-displacement or multichannel)	MLS
Polypropylene tubes (various sizes)	MLS
Vortexer	MLS
Centrifuge	MLS
(Recommended, but optional) One of the following: • Hemocytometer • Countess® Automated Cell Counter	 • MLS • Invitrogen (PN C10227)

Workflow: Prepare samples using the Protein Quant Sample Lysis Kit

Prepare the sample lysis solution (page 25) ↓	
Count (recommended), isolate, and lyse the cells (2500 cells/µL) (page 26) ↓	
(Optional) Determine the total protein concentration (page 26) ↓	
(Optional) Store the cell lysates (page 26)	

About cell sample concentration

TaqMan® Protein Assays relative quantitation experiments are highly dependent on the accurate quantitation of cells or total protein in the test samples.

When using the Protein Quant Sample Lysis Kit to prepare lysates for the TaqMan® Protein Assays kits, Applied Biosystems recommends that you prepare 2500 cells/µL (or 250 ng/uL if quantifying by total protein) per sample. This will be diluted to an initial concentration of 250 cells/µL (or 25 ng/µL) during assay setup. However, you may need to use a higher cell sample concentration to detect low-abundance proteins. Do not prepare lysates with more than 1000 cells/µL (or 100 ng/µL), as excess cellular material may generate false-positive signals in the TaqMan® Protein Assay.

Prepare the sample lysis solution

IMPORTANT! Prepare the sample lysis solution immediately before you lyse the cells. If you are preparing more than one cell lysate sample, you can prepare sufficient sample lysis solution for all of your samples, but you must use the sample lysis solution within 2 hours. Discard any unused solution after 2 hours.

1. Thaw or place the following reagents on ice:
   - Sample Lysis Buffer
   - Protease inhibitor, such as the Calbiochem® Protease Inhibitor Cocktail Set I
   - Phosphatase inhibitor, such as the Calbiochem® Phosphatase Inhibitor Cocktail Set II

   Note: The Calbiochem Protease Inhibitor Cocktail Set I and the Calbiochem Phosphatase Inhibitor Cocktail Set II are not included in the Protein Quant Sample Lysis Kit. The Calbiochem Phosphatase Inhibitor Cocktail Set II is supplied as a 100× solution in water.

2. If necessary, reconstitute the Calbiochem Protease Inhibitor Cocktail Set I to a 100× stock solution:
   a. Add 1 mL of molecular biology grade nuclease-free water to the vial containing the Calbiochem Protease Inhibitor Cocktail Set I (lyophilized powder).
   b. Briefly vortex the reconstituted Calbiochem Protease Inhibitor Cocktail Set I.
   c. Place the vial on ice.

      Note: You can store the reconstituted Protease Inhibitor Cocktail solution at –20°C for repeated use.

3. Combine the components listed below.

Component	Volume (µL)†
Sample Lysis Buffer	1000
Calbiochem® Phosphatase Inhibitor Cocktail Set II, 100×	10
Calbiochem® Protease Inhibitor Cocktail Set I, 100×	10
Total volume of sample lysis solution	**1020**

† When preparing lysates at the recommended concentration of 2500 cells/uL, 1 mL of the sample lysis solution is sufficient to lyse up to 2.5 million cells.

4. Mix gently, then briefly centrifuge the tube.
5. Place the sample lysis solution on ice.

IMPORTANT! After preparing the sample lysis solution, proceed *immediately* to "Count (recommended), isolate, and lyse the cells" on page 26.

Count (recommended), isolate, and lyse the cells

For best results, perform the steps below for one sample at a time, instead of performing the steps for several samples at once.

1. Collect the sample cells according to your laboratory protocol.
2. (Optional, but recommended) Count the cells using, for example, a hemocytometer or the Countess® Automated Cell Counter. (See Appendix B, "Obtaining Cell Counts" on page 57.)
3. Place the following reagents on ice:
   - Sample lysis solution (from page 25)
   - 1✕ PBS, pH 7.4
4. Centrifuge the cells at 500 × g for 4 minutes.
5. Wash the cells twice with ice-cold 1✕ PBS (to remove residual media).
6. Centrifuge the cells again at 500 × g for 4 minutes.
7. Remove all of the supernatant, being careful not to disturb the cell pellet, then place the tube on ice.
8. Add the ice-cold sample lysis solution directly to the cell pellet to a concentration of 2500 cells/µL.
9. Gently disperse and lyse the cells by pipetting up and down several times, then place the cell lysate on ice.

(Optional) Determine the total protein concentration

To determine the total protein concentration, perform protein quantitation using a total protein quantification assay (for example, the Micro BCA™ Protein Assay Kit).

(Optional) Store the cell lysates

Applied Biosystems recommends that you perform the TaqMan® Protein Assays experiment soon after you prepare the cell lysates. If you do not plan on performing the TaqMan® Protein Assays experiment within a few hours after preparing the cell lysates, Applied Biosystems strongly recommends that you store the cell lysates as follows:

1. Divide the prepared cell lysates into aliquots of ~50 µL.
2. Quick-freeze by placing the cell lysate tubes in a dry-ice/ethanol bath.
3. Store the cell lysates at –50°C or below (for example, –70°C).

Section 3 Perform the TaqMan® Protein Assays experiment

This section covers:

User-supplied materials

The materials listed in this section are required to perform TaqMan® Protein Assays experiments, but are not included in the TaqMan® Protein Assays or core reagents kits. Unless otherwise indicated, all items are available from major laboratory suppliers (MLS).

Cell lysates

Perform this procedure with cell lysates that have been prepared with one of the following:

- The Protein Expression Sample Preparation Kit (see page 19)
- The Protein Quant Sample Lysis Kit (see page 23)
- Other commercially available sample lysis reagents (for a list of recommendations, see page 55)

You can also use lysates that are included in the Raji and NTERA2 Protein Expression Lysates Control Kits. See "Control kits" on page 11.

Reagents

Reagent	Source†
Molecular biology grade nuclease-free water	MLS

† For the SDS (Safety Data Sheet) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

General laboratory equipment

Item	Source
Disposable gloves	MLS
Pipette tips (aerosol-resistant)	MLS
Pipettes (positive/air-displacement or multichannel)	MLS
Polypropylene tubes (various sizes)	MLS
Vortexer	MLS
Centrifuge (with plate adapter)	MLS
Microcentrifuge	MLS
For all incubation steps: Thermal cycler with a heated cover **Note:** Applied Biosystems recommends using a thermal cycler for all incubation steps; however, you can use an incubator for the binding and ligation incubation steps.	Applied Biosystems GeneAmp® PCR System 9700, or equivalent
For use with the thermal cycler during the incubation steps: • MicroAmp® Clear Adhesive Film • MicroAmp® Optical Film Compression Pad, 5 pads	 • Applied Biosystems (PN 4306311) • Applied Biosystems (PN 4312639)

Reaction plates and accessories

The table below lists the reaction plates and accessories available for Applied Biosystems real-time PCR systems.

Real-time PCR system (Fast system recommended)	Reaction plates and accessories
7500 Fast system	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film, 100 films (PN 4311971) • MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032)
7900HT/7900HT Fast system, Standard 96-Well Block Module	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • MicroAmp® Optical Adhesive Film, 100 films (PN 4311971) • MicroAmp® Optical Film Compression Pad, 5 pads (PN 4312639) • MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032) • MicroAmp® Snap-On Optical Film Compression Pad, for use with the automation accessory (PN 4333292)
7900HT/7900HT Fast system, 384-Well Block Module	• MicroAmp® Optical 384-Well Reaction Plate with Barcode: – 1000 plates (PN 4343814) – 500 plates (PN 4326270) – 50 plates (PN 4309849) • MicroAmp® Optical 384-Well Reaction Plate, 1000 plates (PN 4343370) • MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)
7900HT/7900HT Fast system, Fast 96-Well Block Module	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film, 100 films (PN 4311971) • MicroAmp® Snap-On Optical Film Compression Pad, for use with the automation accessory (PN 4333292)
StepOnePlus™ system	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)

Workflow: Perform the TaqMan® Protein Assays experiment

Prepare dilutions of each cell lysate (page 33)

↓

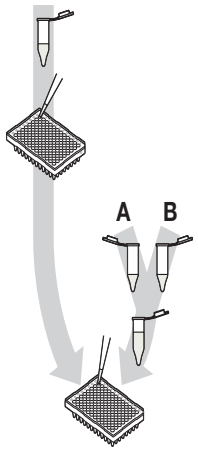
Perform the binding reaction (page 37)

Prepare the Assay Probe solution (page 37)

↓

Prepare and incubate the binding reaction plate (page 38)

↓



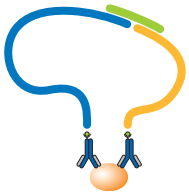
Perform the ligation and protease reactions (page 40)

Perform the ligation reaction (page 40)

↓

Perform the protease reaction (page 42)

↓



Perform the real-time PCR (page 43)

Prepare the PCR mix (page 43)

↓

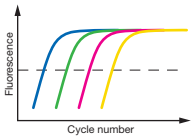
Prepare the PCR plate (page 44)

↓

Run the PCR plate (page 45)

↓

Analyze the real-time data (page 47)



Before you begin

IMPORTANT! For optimal results, follow the recommendations in this section when you perform TaqMan® Protein Assays experiments.

Use correct pipetting techniques

Precise volume delivery, especially during the binding step, is crucial for the performance and reproducibility of TaqMan® Protein Assays experiments. When transferring reagents:

- Use repeat or multichannel pipettes to transfer reagents.
- Verify that all tips are properly seated prior to fluid transfer.
- Use pipettes that are calibrated regularly.
- Pre-aliquot reagents into a separate reaction plate or reservoir for loading into the multichannel pipette. Use new pipette tips for each pipetting step.
- Avoid creating bubbles when pipetting fluids.
- When preparing dilutions or reactions, place the tubes and plates on ice while transferring reagents. Use a plate holder to help stabilize the plate.
- Inspect the pipette tips to ensure consistent reagent aspiration and delivery.

Include a reference sample

To calculate relative quantification of a given target, designate one biological sample as a calibrator or reference sample. The reference sample is a biological control for basal protein expression; the reference sample should have a pre-characterized level of expression. If possible, prepare the reference and experimental lysates at identical cell concentrations.

Include a No Protein Control

For each assay on each plate, use a No Protein Control (NPC or zero-lysate input).

In place of cell lysate, use the buffer that is in included in one of the kits listed below:

Kit	Buffer†
TaqMan® Protein Assays Buffer Kit	Lysate Dilution Buffer
Protein Expression Lysate Control Kit	
Protein Expression Sample Preparation Kit	Cell Resuspension Buffer

† The buffer formulations in each kit are identical; the buffers can be used interchangeably.

The NPC uses the buffer to generate a reference background threshold cycle (C_T) value; this NPC C_T value controls for the non-target ligation signal noise of the TaqMan® Protein Assay. The sample C_T values are subtracted from the NPC C_T value to generate the ΔC_T dataset: $\Delta C_T = C_T(NPC) - C_T(sample)$. The ΔC_T values represent the true target-mediated signal above background. The ΔC_T values are used to determine presence/absence and to compute relative quantification.

Test new Assay Probe pairs

Before using a new Assay Probe pair with your test samples, test each new Assay Probe pair with a 2- or 3-fold serial dilution of a positive control (purified antigen or known positive sample).

- For cell lysates, start with 500 cells or 50 ng of total protein/well.
- For tissue lysates, the initial input can be as high as 1000 ng of total protein/well.
- If the positive control is a purified antigen, start with 200 pg/well.

Prepare dilutions of each cell lysate

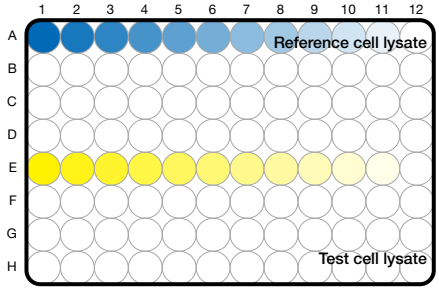
Determine the starting concentrations and minimum lysate dilutions

Applied Biosystems recommends the starting concentrations and minimum lysate dilutions listed below.

Sample preparation kit	Sample	Recommended lysate concentration		Minimum initial dilution	Recommended highest amount per well (2 µL of sample/well)
		Cell count	Protein concentration		
Protein Expression Sample Preparation Kit	Cell lysates	500 cells/µL	NA	1:2	500 cells
Protein Quant Sample Lysis Kit		2500 cells/µL	250 ng/µL	1:10	500 cells or 50 ng
Other sample prep kits (Appendix A on page 55)		2500 cells/µL	250 ng/µL	1:10	500 cells or 50 ng
	Tissue lysates	NA	5000 ng/µL	1:10	1000 ng
NTERA2 and Raji Protein Expression Lysate Control Kits		500 cells/µL (provided)	NA	1:2	500 cells
Recombinant protein in buffer		NA	100,000 pg/mL	None	200 pg

Example plate setup

The example plate setup shown in the figure below is for one reference sample and one test sample, with 2-fold dilutions and four replicates of each dilution. Applied Biosystems recommends either 2-fold or 3-fold dilutions and three or four replicates. Your setup may be different. For suggested alternative plate layouts, see Appendix E on page 67.



Example of a lysate dilution plate setup

IMPORTANT! It is essential to include a No Protein Control (NPC or zero-lysate input) for each pair of Assay Probes used. (For more information, see "Include a No Protein Control" on page 31.)

Prepare the cell lysate dilutions

IMPORTANT! Prepare the cell lysate dilutions on ice.

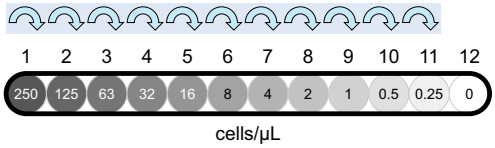
1. Thaw the appropriate buffer and lysates on ice:

If you are using the...	Place the following on ice:
Protein Expression Sample Preparation Kit	Cell Resuspension Buffer†
	Reference and test cell lysates prepared using the Protein Expression Sample Preparation Kit
Protein Quant Sample Lysis Kit	Lysate Dilution Buffer†
	Reference and test cell lysates prepared using the Protein Quant Sample Lysis Kit
Protein Expression Lysate Control Kits (Raji or NTERA2)	Lysate Dilution Buffer†
	Raji or NTER2 Control Lysates from the Protein Expression Lysate Control Kits

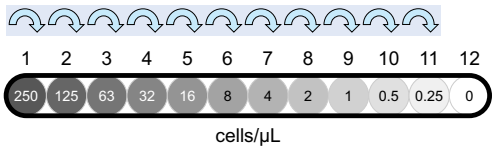
† The buffer formulations in each kit are identical; the buffers can be used interchangeably.

2. Place a 96-well reaction plate on ice.

3. In row A of the reaction plate, prepare 2-fold serial dilutions of the reference cell lysate:

For lysates that require...	Perform the following steps...	Row A example
Initial dilutions of 1:2	1. Add 12 µL of Cell Resuspension Buffer (or Lysate Dilution Buffer) to wells A1 through A12. 2. Add 12 µL of reference cell lysate to well A1. Pipet up and down several times to mix the sample. 3. Continue to transfer 12 µL of reference cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times, until you add lysate to well A11 (A12 contains no lysate).	
Initial dilutions of 1:10	1. Add 18 µL of Lysate Dilution Buffer to well A1. 2. Add 12 µL of Lysate Dilution Buffer to wells A2 through A12. 3. Add 2 µL of reference cell lysate to well A1. Pipet up and down several times to mix the sample. 4. Continue to transfer 12 µL of reference cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times, until you add lysate to well A11 (A12 contains no lysate).	

4. In row E of the reaction plate, prepare 2-fold serial dilutions of the test cell lysate:

For lysates that require...	Perform the following steps...	Row E example
Initial dilutions of 1:2	1. Add 12 µL of Cell Resuspension Buffer (or Lysate Dilution Buffer) to wells E1 through E12. 2. Add 12 µL of test cell lysate to well E1. Pipet up and down several times to mix the sample. 3. Continue to transfer 12 µL of test cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times, until you add lysate to well E11 (E12 contains no lysate).	
Initial dilutions of 1:10	1. Add 18 µL of Lysate Dilution Buffer to well E1. 2. Add 12 µL of Lysate Dilution Buffer to wells E2 through E12. 3. Add 2 µL of test cell lysate to well E1. Pipet up and down several times to mix the sample. 4. Continue to transfer 12 µL of test cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times, until you add lysate to well E11 (E12 contains no lysate).	

5. Briefly centrifuge the plate (for example, 700 × g for 30 seconds) to remove bubbles.

6. Place the plate back on ice.

After diluting the cell lysates, proceed to "Perform the binding reaction" on page 37.

IMPORTANT! The diluted lysates must be used on the day of preparation. Do not store the diluted lysates overnight.

Perform the binding reaction

Compatible Assay Probes

You can use this procedure with the following Assay Probes:

- Assay Probes from any of the TaqMan® Protein Assays kits listed on page 11.
- Assay Probes that you have prepared according to the *TaqMan® Protein Assays Probe Development Protocol*. See "Documentation" on page 73.

Guidelines for preparing the binding reaction

- Follow the guidelines for transferring reagents, as described in "Use correct pipetting techniques" on page 31.
- Inspect the pipette tips to ensure consistent reagent aspiration and delivery. Volume variation generates significant variations in the threshold cycle (C_T) values calculated by the real-time PCR system.
- Do not mix the binding step solutions by pipetting up and down in the binding plate; centrifuge the plate instead.

Prepare the Assay Probe solution

IMPORTANT! Prepare the Assay Probe solution no more than 20 minutes before performing the binding reaction.

1. Place the following reagents on ice:
   - Assay Probe Dilution Buffer
   - Assay Probe A
   - Assay Probe B
2. Mix the reagents completely before use:
   a. When the Assay Probe Dilution Buffer is completely thawed, gently mix the solution.
   b. Gently mix the Assay Probe A and Assay Probe B solutions. Do not vortex the Assay Probes.
3. Combine the components listed below *in the order indicated*.

Order to combine	Component	Volume (µL)
1	Assay Probe Dilution Buffer, 1×	216
2	Assay Probe A, 20×	12
3	Assay Probe B, 20×	12
Total volume of Assay Probe solution†		**240**

† The total volume is sufficient for preparing one 96-well reaction plate.

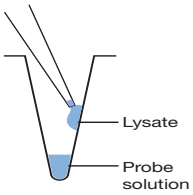
4. Mix gently, then centrifuge the tube (for example, at 700 × g for 30 seconds).

5. Place the Assay Probe solution on ice.

After preparing the Assay Probe solution, proceed to "Prepare and incubate the binding reaction plate" below.

Prepare and incubate the binding reaction plate

Note: It is not necessary to mix the solutions in the binding reaction plate. You can transfer the reaction components to the well walls so that the reagents combine during centrifugation.

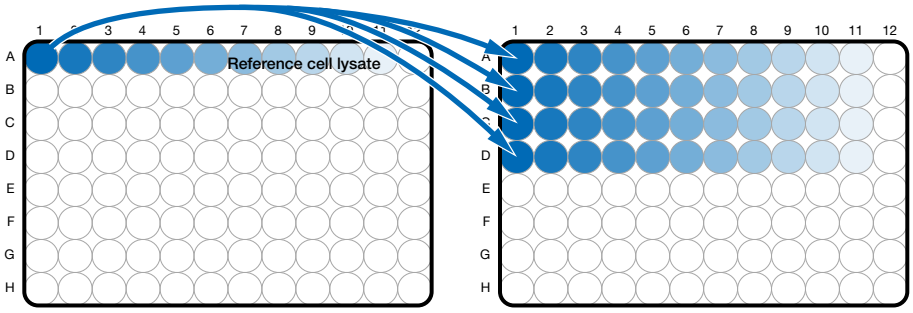


1. Place a 96-well reaction plate on ice.

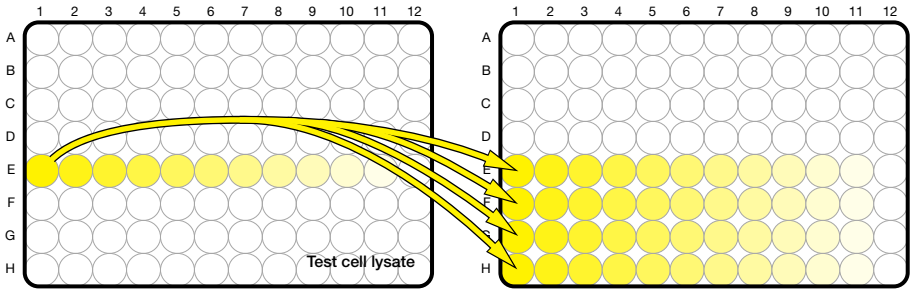
IMPORTANT! Keep the reaction plate on ice while you add the reaction components (step 2 through step 5 below).

2. Add 2 µL of the Assay Probe solution to each well of the reaction plate.

3. Add the reference cell lysate dilutions:
   a. Transfer 2 µL of the diluted reference cell lysate from wells A1 through A12 of the lysate dilution plate to the corresponding wells in row A of the binding reaction plate.
   b. Repeat step a for rows B through D of the binding reaction plate.



4. Add the test cell lysate dilutions:
   a. Transfer 2 µL of the diluted test cell lysate from wells E1 through E12 of the lysate dilution plate to the corresponding wells in row E of the binding reaction plate.
   b. Repeat step a for rows F through H of the binding reaction plate.



5. Seal the binding reaction plate with a MicroAmp® Clear Adhesive Film.

6. Briefly centrifuge the sealed binding reaction plate.

7. Incubate the sealed reaction plate using the thermal-cycling conditions provided in the table below. Be sure to:
   - Use a thermal cycler with a heated cover.
   - Place a MicroAmp® Optical Film Compression Pad on top of the sealed reaction plate to form a tight seal during incubation.

IMPORTANT! To prevent condensation and evaporation, you must use a compression pad and a heated cover when using the thermal cycler. Failure to do so will result in highly variable data.

Stage	Temperature (°C)	Time
HOLD	37	60 minutes
HOLD	4	∞

Keep the binding reactions at 4°C until you are ready to open the binding reaction plate. Applied Biosystems recommends that you proceed to "Perform the ligation and protease reactions" on page 40 within 15 minutes.

Perform the ligation and protease reactions

Guidelines for the ligation and protease reactions

- Follow the guidelines for transferring reagents, as described in "Use correct pipetting techniques" on page 31.
- Inspect the pipette tips to ensure consistent reagent aspiration and delivery. Volume variation generates significant variations in the threshold cycle (C_T) values calculated by the real-time PCR system.
- Use reservoirs and multichannel pipettes to aliquot the ligation and protease reagents.

IMPORTANT! Keep all reagents on ice when not in use. Do not allow the tubes to warm to room temperature.

IMPORTANT! Keep the reaction plates on ice during reagent transfers.

Perform the ligation reaction

1. Thaw or place the following components on ice:
   - DNA Ligase, 500×
   - Ligase Dilution Buffer, 1×
   - Ligation Reaction Buffer, 20×
   - 1× PBS, pH 7.4 (for protease reactions)
   - Protease, 100×
2. Dilute the DNA Ligase:

   IMPORTANT! Prepare fresh diluted ligase for each experiment.

   a. Combine the components listed below.

Component	Volume (µL)
DNA Ligase, 500×	2
Ligase Dilution Buffer, 1×	998
Total volume of diluted DNA Ligase	**1000**

   b. Mix gently.

   c. Place the diluted DNA Ligase on ice.

3. Prepare the ligation solution:
   a. Combine the components listed below.

Component	Volume (µL)	
	For 1 reaction	For 1 plate
Ligation Reaction Buffer, 20X	5.0	600
Nuclease-free water	90.9	10,908
Diluted DNA Ligase (from step 2 above)	0.1	12
Total volume of ligation solution	**96.0**	**11,520**

   b. Invert the tube to mix the components.
   c. Place the ligation solution on ice.

4. Add the ligation solution to the binding reaction plate:
   a. Remove the binding reaction plate from the thermal cycler.
   b. Remove the MicroAmp® Clear Adhesive Film from the binding reaction plate, then place the plate on ice.
   c. Add 96 µL of the ligation solution to each well in the binding reaction plate. Pipet up and down once to mix.

5. Using a new MicroAmp Clear Adhesive Film, reseal the ligation reaction plate.

6. Briefly centrifuge the sealed reaction plate.

7. Incubate the sealed reaction plate using the thermal-cycling conditions provided in the table below. Be sure to:
   - Use a thermal cycler with a heated cover.
   - Place a MicroAmp® Optical Film Compression Pad on top of the sealed reaction plate to form a tight seal during incubation.

IMPORTANT! To prevent condensation and evaporation, you must use a compression pad and a heated cover when using the thermal cycler. Failure to do so will result in highly variable data.

Step	Cycle number	Temperature (°C)	Time	Reaction volume
Ligation	1	37	10 minutes	Default
Cooling	1	4	Up to 10 minutes	Default

After performing the ligation reaction, proceed to "Perform the protease reaction" on page 42.

Note: You can omit the protease step if you proceed *immediately* to the real-time PCR step on page 43. Otherwise, continue with the protease reaction on page 42.

Perform the protease reaction

1. Dilute the protease:
   a. Briefly vortex the protease to mix the solution.
   b. Combine the components listed below.

Component	Volume (µL)
Protease, 100×	4
1× PBS, pH 7.4	396
Total volume of diluted protease solution	**400**

   c. Mix gently, then briefly centrifuge to spin the liquid to the tube bottom.
   d. Place the diluted protease solution on ice.

2. Add the diluted protease solution to the ligation reaction plate to terminate the ligation reaction:
   a. Remove the ligation reaction plate from the thermal cycler.
   b. Remove the MicroAmp Clear Adhesive Film from the ligation reaction plate, then place the plate on ice.
   c. Add 2 µL of the diluted protease to each well of the ligation reaction plate.

      Note: No mixing is required. The protease will diffuse throughout the samples during the 10-minute incubation.

3. Using a new MicroAmp Clear Adhesive Film, reseal the reaction plate.

4. Incubate the sealed reaction plate using the thermal-cycling conditions provided in the table below. Be sure to:
   - Use a thermal cycler with a heated cover.
   - Place a MicroAmp® Optical Film Compression Pad on top of the sealed reaction plate to form a tight seal during incubation.

IMPORTANT! To prevent condensation and evaporation, you must use a compression pad and a heated cover when using the thermal cycler. Failure to do so will result in highly variable data.

Step	Cycle number	Temperature (°C)	Time	Reaction volume
Terminate ligation	1	37	10 minutes	Default
Inactivate protease	1	95	5 minutes	Default
HOLD	1	4	Hold	Default

5. Remove the reaction plate from the thermal cycler and place it on ice.

After performing the protease reaction:

- Proceed to "Perform the real-time PCR" below.

  OR
- Store the protease-treated ligation products at 4°C for up to 3 days, or at –20°C for up to 2 weeks.

Perform the real-time PCR

Guidelines for performing the real-time PCR

- Follow the guidelines for transferring reagents, as described in "Use correct pipetting techniques" on page 31.
- Power on your real-time PCR instrument at least 30 minutes before loading the PCR reaction plate.
- (For the 7900HT/7900HT Fast systems) Be sure that the plate-loading door is closed until just before you load the PCR reaction plate.

IMPORTANT! Keep all reagents on ice when not in use. Do not allow the tubes to warm to room temperature.

IMPORTANT! Keep the reaction plates on ice during reagent transfers.

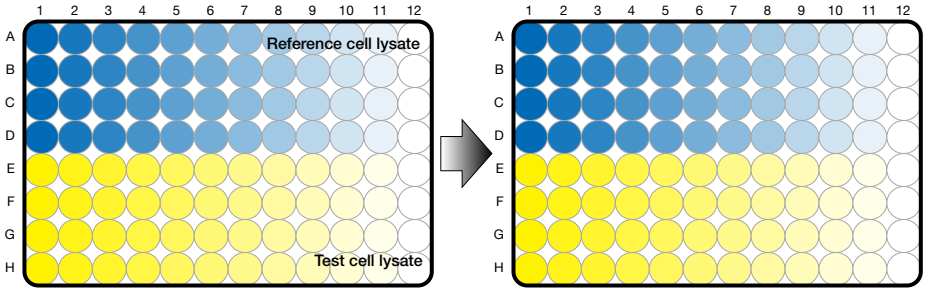
Prepare the PCR mix

1. Thaw the Universal PCR Assay on ice.
2. Combine the components listed below.

Component	Volume (µL)	
	For 1 reaction	For 1 plate
Fast Master Mix, 2X	10	1200
Universal PCR Assay, 20X	1	120
Total volume of PCR mix	**11**	**1320**

3. Mix gently, then briefly centrifuge to spin the liquid to the tube bottom.
4. Place the PCR mix on ice.

Prepare the PCR plate



Protease-treated reaction plate PCR plate

1. Place a PCR plate on ice.
2. Add 11 µL of the PCR mix to each well of the PCR plate.
3. Add the protease-treated ligation product to the PCR plate:
   a. Remove the MicroAmp® Clear Adhesive Film from the protease reaction plate, then place the plate on ice.
   b. Transfer 9 µL of the protease-treated ligation product from each well of the protease reaction plate to each corresponding well of the PCR plate. When transferring, pipet up and down once to mix.
4. Seal the PCR plate with a MicroAmp® Optical Adhesive Film (do not use a standard adhesive cover).
5. Briefly centrifuge the PCR plate to combine the solutions and remove bubbles.

   If you perform the run on 7900HT/7900HT Fast system with a 96-Well Block Module and an automation accessory, place a MicroAmp® Snap-On Optical Film Compression Pad on top of the plate.

IMPORTANT! After preparing the PCR plate, proceed *immediately* to "Run the PCR plate" on page 45.

Run the PCR plate

1. In your real-time PCR system software, create a plate document/experiment for the run. Use the setup information provided in the table below.

   Note: Table 1 on page 46 provides detailed thermal-cycling conditions for each run type listed below.

System	StepOnePlus™	7500 Fast		7900HT Fast	7900HT
Software	StepOne™ Software v1.0 or later	SDS Software v1.4 or later	7500 Software v2.0 or later	SDS Software v2.1 or later	SDS Software v2.0 or later
Template	cDNA	—	cDNA	—	—
Run type	Fast				Standard
Reaction plate	Fast 96-well			Fast 96-well, Standard 96-well, or 384-well	Standard 96-well or 384-well
Sample volume	20 µL				20 µL
Detectors/ targets	Reporter: FAM™ dye Quencher: Non-fluorescent				Reporter: FAM™ dye Quencher: Non-fluorescent
Ramp speed/ mode	Fast			Fast or Standard	Standard
Experiment type	Select an experiment type that will generate C_T values, such as Absolute Quantitation or Standard Curve.				
Tasks and quantities	You do not need to assign tasks or quantities.				
Analysis settings	Threshold cycle (C_T): 0.2 Baseline: Automatic				

2. (For the 7900HT/7900HT Fast systems) Be sure that the plate-loading door is closed until just before you load the PCR reaction plate.

3. Load the PCR plate into your real-time PCR instrument.

4. Start the run.

Table 1 Thermal-cycling conditions

Run type	Reaction plate	Stage	Temperature (°C)	Time (enzyme activation, denaturation, annealing/extension)
StepOnePlus™ system				
Fast	Fast 96-well	Hold	95	20 seconds
		Cycle	95	1 second
		(40 cycles)	60	20 seconds
7500 Fast system				
Fast	Fast 96-well	Hold	95	20 seconds
		Cycle	95	3 seconds
		(40 cycles)	60	30 seconds
7900HT Fast system				
Fast	Fast 96-well	Hold	95	20 seconds
		Cycle	95	1 second
		(40 cycles)	60	20 seconds
7900HT system				
Standard	Standard 96-well or 384 well	Hold	95	2 minutes
		Cycle	95	15 seconds
		(40 cycles)	60	1 minute

Analyze the real-time data

Analyze the data from TaqMan® Protein Assays experiments using the real-time PCR system software. For further data analysis, you can use a spreadsheet program or the ProteinAssist™ Software.

Using the real-time PCR system software

For all real-time PCR systems:

1. View the amplification plots for the entire reaction plate.
2. Analyze the plate run using a threshold cycle (C_T) setting of 0.2 and automatic baseline.

Using a spreadsheet program

1. Export the results from the instrument software to a spreadsheet program (such as Microsoft® Excel® software).
2. Calculate the average C_T values for each sample.
3. Calculate the ΔC_T values for each sample:
   $AvgC_T(NPC) – AvgC_T(sample)$
4. Plot the ΔC_T values *vs.* cell input on a semi-log graph.

Using the ProteinAssist™ Software

Compatible real-time PCR system software

The ProteinAssist™ Software can be used for experiments generated with the following real-time PCR system software:

- **7500 Fast system** – SDS Software v1.4 or later and 7500 Software v2.0.2 or later
- **7900HT Fast system** – SDS Software v2.3 or later
- **StepOnePlus™ system** – StepOne™ Software v2.1 or later
- **ViiA™ 7 Real-Time PCR System** – ViiA™ 7 Software v1.0 or later

Determine relative protein expression

To determine the relative protein expression of a given target between different samples:

1. Download the ProteinAssist Software from:
   www.appliedbiosystems.com/taqman4protein
2. Refer to the Getting Started Guide for instructions on how to import and analyze C_T data. (To open the Getting Started Guide, click the **Help** tab in the software.)

For additional information about TaqMan® Protein Assay data analysis, refer to the *Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide*. See "Documentation and Support" on page 73.

Expected results

Use the table below as a guide to determine if the Assay Probes are binding to their target antigen in the TaqMan® Protein Assay. From day-to-day, the absolute C_T values for both the NPC and the sample may shift up or down. However, the ΔC_T value [AvgC_T(NPC) – AvgC_T(sample)] should remain constant.

ΔC_T value	Comment	
≥3	As a general rule, an assay that yields ΔC_T values of 3 and above and can produce a typical dose-response curve.	
<3	**Recommended actions**	Another source or preparation of biotinylated antibody should be considered only when the ΔC_T value is <3 *and*: • The biotinylated antibody has passed the Forced Proximity Probe Test. • The positive sample is a validated control. • User-related error has been eliminated from consideration.

For more information

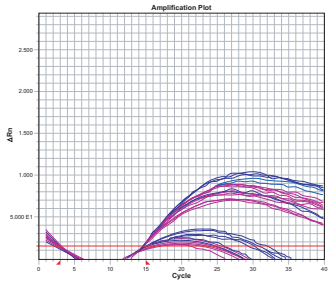
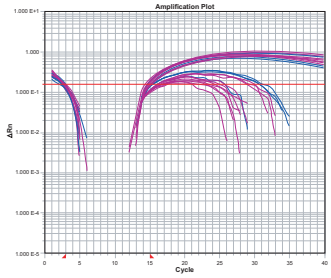
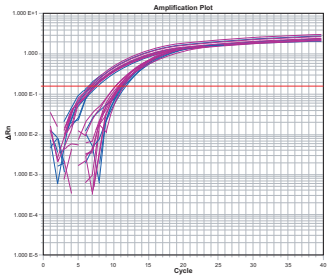
For more information on:

- **Data analysis** – Refer to the *Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide* and to your real-time PCR system documentation.
- **Relative quantitation** – Refer to the Help system in the ProteinAssist Software.

For document part numbers and instructions on accessing the software Help system, see "Documentation and Support" on page 73.

Section 4 Troubleshooting

Troubleshooting

Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔR_n values Linear view:  Log view:  	The baseline was set improperly (some samples have C_T values lower than the baseline stop value)	Refer to your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baseline, or move the baseline stop value to a lower C_T (2 cycles before the amplification curve for the sample crosses the threshold). Log view corrected: 
Amplification curve shows weak amplification	Degraded reagents and/or probe	• Check the expiration date of the reagents. • Verify that you followed the correct handling and storage conditions. • Avoid excessive freeze-thaw cycles.
	Primer-dimer formation and residual polymerase activity	(For optimal results, run the reaction plate immediately after completing the reaction setup. If you cannot run a reaction plate within 2 hours after completing the reaction setup, refrigerate or freeze the reaction plate until you can run it.
Amplification curve shows low ROX™ dye (passive reference dye)	Inaccurate pipetting: Little or no TaqMan® Protein Expression Fast Master Mix	Follow accurate pipetting practices.

Troubleshooting

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_T = 40) across all assays or in an unusually large number of assays	One or more of the reaction components was not added	Verify that the Assay Probes, Ligase, Ligation Reaction Buffer, Universal PCR Assay, and TaqMan® Protein Expression Fast Master Mix were added to the reaction plates. (If the master mix is missing, the passive reference fails.)
	Incorrect dye components were selected	Check the dye components settings and reanalyze the data.
	The annealing temperature on the thermal cycler was too high for the primers and/or probe	Verify that the thermal cycler is set to the correct annealing and extension temperatures. Verify that the thermal cycler is calibrated and maintained regularly.
	PCR inhibition	• Rerun the assay with fresh lysate prepared with freshly prepared protease and phosphatase inhibitors. • Use the Protein Expression Lysate Control Kit as a positive control in order to troubleshoot.
	The baseline and/or threshold was set improperly	Applied Biosystems recommends that you use an automatic baseline and threshold setting of 0.2.
	Sample evaporation occurred during the binding reaction	When using a thermal cycler for the binding reaction, use a heated cover and a MicroAmp® Optical Film Compression Pad.
	Before the real-time PCR reaction, the protease was not completely inactivated	When using a thermal cycler for the protease reaction, use a heated cover and a MicroAmp® Optical Film Compression Pad.
Amplification curve shows samples within the same assay that have differently shaped curves	Incorrect sample was used	Rerun the assay.
	Sample quality is poor	Rerun the assay with freshly prepared lysate.
	Imprecise pipetting: different concentrations	Follow accurate pippetting practices.
	Contamination	Be sure your workspace and equipment are properly cleaned.
Decrease in ROX™ dye fluorescence (passive reference dye)	Precipitation in the TaqMan® Master Mix	Be sure to mix the reagents well.
	Degraded TaqMan® Master Mix	Verify that the kits have been stored according to the instructions on the packaging and have not expired.
Simultaneous increase in fluorescence from both the: • Passive reference (ROX™ dye) • Reporter dye(s)	Evaporation occurred during PCR	Check the volumes in each well. Check the seal of the MicroAmp® Optical Adhesive Film for leaks.
Multicomponent signal for ROX™ dye is not linear	Pure dye components spectra are incorrect	Rerun the pure dye spectra.
	Incorrect dye components were selected	Select the correct dyes for the data analysis.

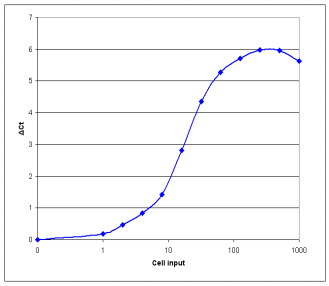
Troubleshooting

Observation	Possible cause	Recommended action
R_n on R_n-vs.-Cycle plot is very high	ROX™ dye was not selected as the passive reference when the plate document/ experiment were set up	Select the ROX dye as the passive reference, then reanalyze the data.
Inconsistent data (high standard deviation of replicates, C_T varies)	Inefficient mixing of reagents	• Increase the length of time that you mix the reagents. • Check your mixing process by running a replicate plate.
	Inaccurate pipetting	Check the calibration of the pipettes.
	The threshold and/or baseline was set improperly	Use a threshold cycle (C_T) of 0.2 with automatic baseline.
	Sample evaporation occurred during the binding reaction	When using a thermal cycler for the binding reaction, use a heated cover and a MicroAmp® Optical Film Compression Pad.
	Before the real-time PCR reaction, the protease was not completely inactivated	When using a thermal cycler for the protease reaction, use a heated cover and a MicroAmp® Optical Film Compression Pad.
	Amplicon contamination occurred	• Follow good laboratory practices (see page 63). • When removing the MicroAmp® Clear Adhesive Film, avoid splashes between wells. Remove the adhesive film before placing the reaction plate on ice; the adhesive film is easier to remove when the plate is still warm.
	Low concentration of target	Rerun the assay using lysate prepared with higher cell count.
C_T value for sample is lower than expected	More lysate added than expected	Reduce the amount of lysate. Applied Biosystems recommends a maximum of 500 cells/reaction.
	Template or amplicon contamination	Follow good laboratory practices (see page 63).

Troubleshooting

Observation	Possible cause	Recommended action
Background and/or sample C_T are lower than expected	Excess Assay Probes or ligation components	Rerun the assay using the correct amounts of components.
	Biotinylated antibody aggregation occurred	Follow the antibody guidelines in the *TaqMan® Protein Assays Probe Development Protocol* to minimize antibody aggregation. See "Documentation" on page 73.
	The Assay Probes were made with an excess of Prox-Oligos	Remake the Assay Probes using the correct amount of Prox-Oligos.
	The Antibody Dilution Buffer, Cell Resuspension Buffer, Lysate Dilution Buffer, and/or Assay Probe Storage Buffer were improperly handled	Avoid: • Over-mixing • Over-vortexing • Multiple freeze-thaw cycles See "Kit contents and storage" on page 11.
	Incomplete or no ligase inactivation occurred	• If the protease step was included, rerun the assay using the correct amount of protease. • If the protease step was not included, rerun the assay, being sure to perform the PCR reaction immediately after the ligation reaction.
Background and/or sample C_T are higher than expected	Insufficient Assay Probes or ligation components	Rerun the assay using the correct amounts of components.
	The biotinylated antibody is at a lower-than-expected concentration	1. Verify the concentration of the biotinylated antibody. 2. Remake the Assay Probes with the correct amount of biotinylated antibodies and Prox-Oligos.
	The Assay Probes were incorrectly made	
	The ligase is too dilute	Rerun the assay using the correct amount of components.
	The ligase has become inactivated	Rerun the assay with freshly diluted ligase.
	Before the real-time PCR reaction, the protease was not completely inactivated	When using a thermal cycler for the protease reaction, use a heated cover and a MicroAmp® Optical Film Compression Pad.
Sample C_T is similar to background C_T	Lysate contains insufficient or no target protein or protein has degraded	• Rerun the assay using more cells/µL. • Rerun the assay using freshly prepared lysate with protease and phosphatase inhibitors • Use a different sample lysis reagent.
	Mismatched Assay Probes	Rerun the assay using the correct Assay Probes.
Shifting R_n value during the early cycles of the PCR (cycles 0 to 5)	Fluorescence did not stabilize to the buffer conditions of the reaction mix **Note:** This condition does not affect PCR or the final results.	Use automatic baseline.

Troubleshooting

Observation	Possible cause	Recommended action
Small ΔR_n	PCR efficiency is poor	Repeat the PCR reaction.
	Quantity of target is low	Increase the quantity of the target by using more cells/µL.
Noisy signal above the threshold	Evaporation occurred during PCR	• Check the seal of the MicroAmp® Optical Adhesive Film for leaks, and check the volumes in each well. • If you perform the run on 7900HT/7900HT Fast system with a 96-Well Block Module and an automation accessory, place a MicroAmp® Snap-On Optical Film Compression Pad on top of the plate.
	Empty well due to inaccurate pipetting	Check the calibration of the pipettes.
	The well is labeled with a detector in the plate document/experiment, but the well is empty	• Be sure your plate document/experiment are set up correctly. • Exclude the well and reanalyze the data.
Lower than expected ΔC_T values at higher cell inputs 	This is expected when the target is in excess of the Assay Probes	No action required; normal occurrence. For more information, refer to the *Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide.* See "Documentation" on page 73.

Sample Lysis Reagents

Recommended reagents

You can use other commercially available sample lysis reagents to prepare cell lysates for use with the TaqMan® Protein Assays. Applied Biosystems recommends the reagents listed in the table below.

	Applied Biosystems (kits developed for TaqMan® Protein Assays)		Invitrogen					Ambion		Biochain
Product	Protein Expression Sample Lysis Kit	Protein Quant Sample Lysis Kit	Cell Extraction Buffer	NP40 Cell Lysis Buffer	Denaturing Cell Extraction Buffer	Tissue Extraction Reagent I	NativePAGE™ Sample	PARIS™ Kit	mirVana™ PARIS™ Kit	Total Protein Extraction Kit: TM Buffer
Part number	4405443	4448536	FNN0011	FNN0021	FNN0091	FNN0071	BN2008	AM1921	AM1556	K3011010-1
Initial dilution†	1:2	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10

† This is the initial dilution required before using the lysate in the TaqMan Protein Assays experiment.

Component compatibility table

Component	Concentration in the lysis reagent†
Triton X-100	1%
NP40	1%
DDM (n-dodecyl-beta-D-maltoside)	2%
Digitonin	2%
Sodium dodecyl sulphate	0.1%
Deoxycholate	0.5%
Tris, pH 7.4 to 8	50 mM
Bicine, pH 7.5	25 mM
Sodium chloride	250 mM
Glycerol	1%

† Requires a 1:10 initial dilution before using in the TaqMan Protein Assays experiment.

Obtaining Cell Counts

This appendix covers:

About obtaining cell counts of intact cells

Because TaqMan® Protein Assays are normalized to cell input, it is essential to obtain an accurate cell count for each sample. Obtaining counts of intact cells before preparing the cell lysate is highly recommended.

Cell harvesting

Use your laboratory's procedure for harvesting your cell line. Typical cell harvesting procedures involve scraping or tryspin digestion. You may need to optimize the harvesting procedure to obtain maximal amounts of intact cells.

Cell counts of intact cells

Applied Biosystems recommends counting cells using a hemocytometer or the Countess® Automated Cell Counter. With either counting method, make sure your cell count includes only live, intact cells.

Use a hemocytometer

A hemocytometer provides a standard, reliable method for counting your cell samples before cell lysis. Follow the manufacturer's instructions.

Use the Countess® Automated Cell Counter

The Countess Automated Cell Counter enables automatic counting of intact cells using trypan blue staining combined with a sophisticated image analysis algorithm. For more information, search for **Countess** at **www.invitrogen.com**

Relative cell counts using TaqMan® RNase P Detection Reagents

Note: This method has only been qualified with the Protein Expression Sample Preparation Kit.

Overview

When performing the sample preparation procedures in this protocol, Applied Biosystems recommends that you obtain cell counts on intact cells before lysis. However, if counting intact cells before lysis is not feasible, the cell equivalent concentration of a lysate can be estimated through its genomic DNA (gDNA) concentration, using TaqMan® RNase P Detection Reagents (FAM™ dye; PN 4316831). For lysates prepared and treated in the same manner, the cell equivalent concentration of a lysate relative to the other cell lysates can be used for data normalization or for adjustment of cell lysates to equivalent cell inputs.

This method can be useful in the following situations:

- When the number of cells in your sample is too low to ensure accurate cell counts using a hemocytometer.
- To enable adjustment of cell lysates to equivalent cell input for TaqMan® Protein Assays, for experiments with large numbers of samples, such as screening experiments.

TaqMan® RNase P Detection Reagents are used for detection and quantitation of genomic copies of the human RNase P gene using the 5′ nuclease assay. The human RNase P gene encodes the RNA moiety for the RNase P enzyme, and it is normally a single-copy gene.

IMPORTANT! The Protein Expression Sample Preparation Kit is designed to produce a crude cell lysate optimized for detection of intact proteins, and recovery of gDNA has not been validated. Therefore, quantitation of genomic copies of the RNase P gene should ***not*** be used to determine an absolute cell count in cell lysates.
The method described in this section is appropriate only for comparing samples that have been treated and stored in the same way (for instance, all fresh or all frozen lysates), to enable adjustment of different cell lysates to equivalent concentrations.

Procedure overview

1. Treat a cell lysate (previously prepared using the Protein Expression Sample Preparation Kit) and Human Genomic Control DNA with protease.
2. Prepare serial dilutions of the protease-treated cell lysate and Human Genomic Control DNA.
3. Perform real-time PCR targeting RNase P DNA on the serial dilutions.
4. Calculate the cell concentration in the lysate of interest by comparison to a standard curve prepared from the Human Genomic Control DNA data.

Required materials

The table below lists the required materials for cell count estimates using TaqMan® RNase P Detection Reagents.

Material	Source
Cell lysate of interest, estimated at 100 to 500 cells/µL	Prepared as described in this protocol (page 19 or page 23)
Protease, 100×	TaqMan® Protein Assays core reagents (see page 12 for part numbers)
• Human Genomic Control DNA, 10 ng/µL† • 20× RNase P Primer-Probe (FAM™ dye) Mix	TaqMan® RNase P Detection Reagents (FAM™ dye; PN 4316831)
1× TE (10 mM Tris, pH 8, 1 mM EDTA)	Major laboratory suppliers
TaqMan® Protein Assays Fast Master Mix, 2× (100 rxn)	Applied Biosystems (PN 4400088 or 4448616)
Thermal cycler, such as ABI 9700, and accessories	Applied Biosystems
Real-time PCR instrument and accessories	Applied Biosystems

† A cell lysate prepared from a human cell line whose cell count and copy number of the RNase P gene are known may be substituted.

Procedure

For detailed instructions on handling PCR plates and instrument setup, follow your real-time PCR instrument guide.

Treat 100 µL of cell lysate and control gDNA with 1 µL of Protease

Use nuclease-free, protease-free tubes that are compatible with your thermal cycler.

1. Prepare 100 µL of a 0.5 ng/µL stock of Human Genomic Control DNA: mix 5 µL of Human Genomic Control DNA with 95 µL of 1× TE.
2. Transfer 100 µL of the cell lysate to a new tube.
3. Add 1 µL of 100× Protease to the diluted Human Genomic Control DNA and to the cell lysate.

   You can treat less cell lysate, and scale down the amount of Protease accordingly. If necessary, dilute the Protease in 1× PBS, pH 7.4.
4. Place the tubes in a thermal cycler programmed as follows and start the run.

Cycle	Temperature (°C)	Time
1	37	20 minutes
1	95	5 minutes
–	4	HOLD

Prepare serial dilutions of the lysate and control gDNA

1. Prepare a 1:4 dilution of the protease-treated cell lysate (from page 59) in 1X TE. (For example, mix 10 µL of cell lysate with 30 µL of 1X TE.)
2. Prepare 2- to 4-fold serial dilutions of the diluted lysate (from step 1) in 1X TE, in 6 steps. Prepare ~30 µL of each dilution to provide sufficient sample for triplicate PCR reactions. (A 6-step dilution series should cover the expected concentration range of the cell lysates.)
3. Prepare 2-fold serial dilutions of the protease-treated Human Genomic Control DNA (from page 59) in 1X TE, in 6 steps (see the table in step 2 on page 61). Prepare ~30 µL of each dilution.

   This dilution series may be adjusted according to the expected gDNA concentration of the lysate of interest.

Perform real-time PCR targeting RNase P

1. Prepare RNase P PCR mix as follows, sufficient for triplicate reactions for each dilution point plus No Template Controls (NTC).

Component	Volume (µL)	
	1 reaction	50 reactions
TaqMan® Fast Master Mix, 2X	10	500
20X RNase P Primer-Probe (FAM™ dye) Mix	1	50
Total volume of RNase P PCR mix	**11**	**550**

2. Add 11 µL of RNase P PCR mix to the wells of a reaction plate appropriate for your real-time PCR instrument.
3. Add 9 µL of each gDNA dilution, cell lysate dilution, or 1X TE (for the NTC).

   Note: Set up triplicate PCRs for each dilution, and include NTC wells on each plate.

4. Load the plate into your real-time PCR instrument, and run the plate using the parameters below.
   - Reaction volume – 20 µL
   - Reporter – FAM™ dye
   - Quencher – TAMRA™ dye
   - Plate document/experiment parameters:

System Software	Ramp speed and run settings	Reaction plate	Stage	Temp (°C)	Time
StepOnePlus™ System StepOne™ Software v1.0 or later	Fast	Fast 96-well	Hold	95	20 sec
			Cycle (40 cycles)	95	1 sec
				60	20 sec
7900HT Fast SDS Software v2.1 or later	Fast	Fast 96-well	Hold	95	20 sec
			Cycle (40 cycles)	95	1 sec
				60	20 sec
7900HT SDS Software v2.0 or later	Standard	Standard 96-well or 384-well	Hold	95	2 min
			Cycle (40 cycles)	95	15 sec
				60	1 min
7500 Fast SDS Software v1.4 or later	Fast	Fast 96-well	Hold	95	20 sec
			Cycle (40 cycles)	95	3 sec
				60	30 sec

Calculate cell equivalents

Note: The examples shown in this section assume that the TaqMan® RNase P assay is performed using the same volume of gDNA and cell lysate dilutions. (Adjust for different volumes if necessary.)

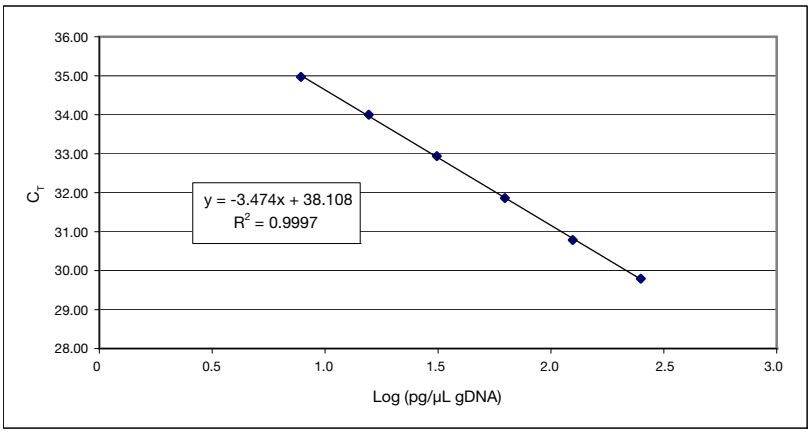
1. Export the C_T data into a spreadsheet program to perform the following calculations.

2. Prepare a standard curve from the Human Genomic Control DNA data: plot C_T vs. log(gDNA concentration), and calculate the linear trend equation.

C_T values of Human Genomic Control DNA dilution series

Dilution #	gDNA conc. (pg/µL)	Log (gDNA conc.)	Avg C_T
1	250.0000	2.40	29.79
2	125.0000	2.10	30.79
3	62.5000	1.80	31.86
4	31.2500	1.49	32.94
5	15.6250	1.19	34.00
6	7.8125	0.89	34.97

As shown in the figure below (standard curve with Human Genomic Control DNA), the slope of the linear trend equation should be about –3.3.



3. Use the linear trend equation calculated in step 2 to calculate the gDNA concentration in the cell lysate of interest.

   For human diploid cells:

   - 6.6 pg of gDNA is equivalent to one cell.
   - 1 ng of gDNA is equivalent to 151.5 cells.

   In the example calculations shown in the table below, the standard curve (shown in the figure in step 2) was used to convert the C_T values from a dilution series of an NTERA2 cell lysate (1 copy of the RNase P gene per cell) to the cell equivalent concentration.

Cell quantitation of NTERA2 cell lysate using TaqMan® RNase P Detection Reagents

Cell extract dilution	Avg C_T	Log(gDNA conc.)†	gDNA conc. (pg/µL)	Calculated lysate conc. (cell eq./µL)‡
1:4	29.28	2.54	347.6	105.3
1:8	30.56	2.17	149.3	45.2
1:16	31.50	1.90	79.9	24.2
1:32	32.36	1.65	45.2	13.7
1:64	33.28	1.39	24.5	7.4
1:128	34.48	1.04	11.1	3.4

† $(C_T - 38.108)/(-3.474)$; linear trend equation shown in the figure in step 2.
‡ NTERA2 cells are haploid at the RNAse P locus, therefore 3.3 pg of gDNA is equivalent to 1 cell.

Recommended Laboratory Practices

PCR assays require special laboratory practices to avoid false positive amplifications. The high-throughput and repetition of these assays can lead to amplification of one DNA molecule.

Good laboratory practices

When preparing samples for PCR amplification:

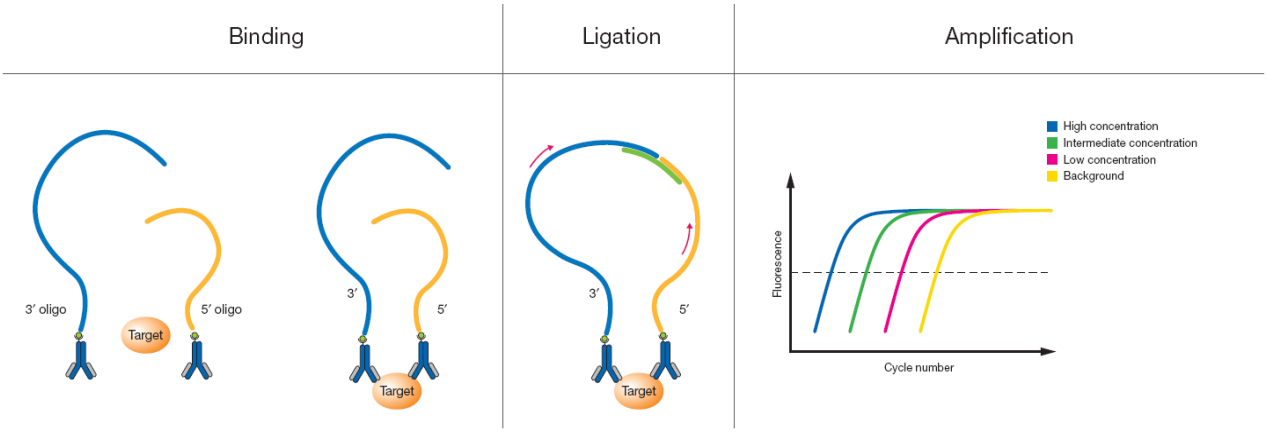
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Chemistry Overview

How TaqMan® Protein Assays work

TaqMan® Protein Assays enable relative quantitation analysis of specific target proteins in cultured cell lysates to be performed on Applied Biosystems real-time PCR systems. The analysis provides a comparative measure of a target protein in a test lysate relative to the abundance of the same target in a reference cell lysate.

TaqMan® Protein Assays enable identification and relative quantitation of target protein in a three-step process:



Binding

During binding, a set of Assay Probes is introduced to the reference and test cell lysates. Each probe consists of a single-stranded oligonucleotide bound (through a streptavidin-biotin linkage) to an antibody that recognizes an epitope of the target protein. When combined in solution, the antibody portions of the probes bind to the corresponding epitopes on the target protein.

Ligation

During ligation, the samples are exposed to a third oligonucleotide that hybridizes to the oligo ends of the Assay Probes. The hybrid structure forms preferentially only when the Assay Probes are bound to the same protein and are consequently in proximity to each other. DNA ligase then covalently links the hybridized ends of the Assay Probes to form a continuous DNA molecule linking the bound antibodies. After the ligation reaction, a protease that inactivates the ligase is added to the samples.

Amplification

The samples are then combined with TaqMan® Protein Assays Fast Master Mix and TaqMan® Universal PCR Assay. The TaqMan® probe binds to a target sequence on the Prox-Probe A oligo sequence.

The amplicon region of the ligated oligonucleotide chains is amplified by PCR performed on an Applied Biosystems real-time PCR system. During the PCR, the instrument records the fluorescence generated by the cleavage of FAM™ dye-labeled MGB probe through the 5′ nuclease assay.

Suggested Alternative Plate Layouts

You can use a variety of plate layouts with the TaqMan® Protein Assays. For example, you can use a single plate to perform relative quantitation of protein expression in more than one test cell lysate. Figures 1 through 3 show examples for two, three, or four test cell lysates on one plate with one set of Assay Probes. It is important to include a No Protein Control (NPC or no lysate input) for each pair of Assay Probes that you use. You can also test multiple targets on the same plate.

Figure 1 Suggested plate layout with two test cell lysates

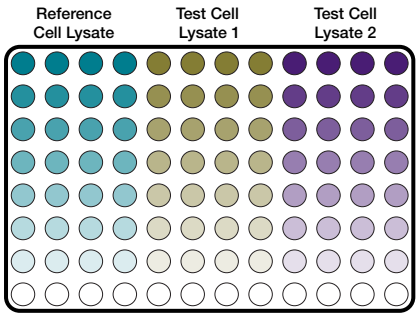


Figure 2 Suggested plate layout with three test cell lysates

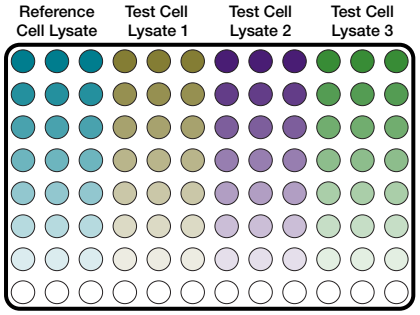
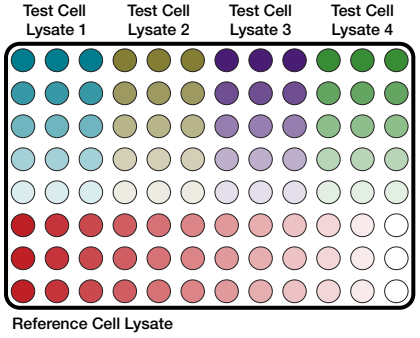


Figure 3 Suggested plate layout with four test cell lysates



Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 70.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (**www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Documentation and Support

Documentation

TaqMan® Protein Assays documentation

Portable document format (PDF) versions of the documents listed below are available at **www.appliedbiosystems.com**

Note: To open the PDF versions, use the Adobe Acrobat Reader software available from **www.adobe.com**

Document	Part number
TaqMan® Protein Assays Sample Prep and Assay Protocol	4449283
TaqMan® Protein Assays Sample Prep Quick Reference Card	4449771
TaqMan® Protein Assays Assay Quick Reference Card	4449281
TaqMan® Protein Assays Probe Development Protocol	4449282
TaqMan® Protein Assays Probe Development Quick Reference Card	4449772
Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide	4405780

Instrument documentation

To obtain the documents listed in this section or additional documentation, see "Obtaining support" on page 74.

7900HT/7900HT Fast system (Fast 96-Well, Standard 96-Well, or 384-Well Block Modules)

Document	Part number
Applied Biosystems 7900HT Fast Real-Time PCR System Quick Reference Card: Performing Fast Gene Quantification	4351892
Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantification Getting Started Guide	4364014
Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4369584
Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4352533

7500 Fast system

Document	Part number
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide	4347824
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems Absolute Quantification Getting Started Guide	4347825
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments	4387779
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Comparative C_T/Relative Standard Curve Experiments	4387783

StepOnePlus™ system

Document	Part number
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide	4379704
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_T Experiments Getting Started Guide	4376785
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments	4376784

Chemistry/reagent guides

Document	Part number
Real-Time PCR Systems Chemistry Guide: Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems	4348358
Applied Biosystems Real-Time PCR Systems Reagent Guide	4387787

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Obtaining information from the Help system

The ProteinAssist™ Software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the ProteinAssist Software window.
- Select **Help**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

Part Number 4449283 Rev. C 02/2011

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Technical Resources and Support
For the latest technical resources and support information for all locations, please refer to our Web site at
www.appliedbiosystems.com