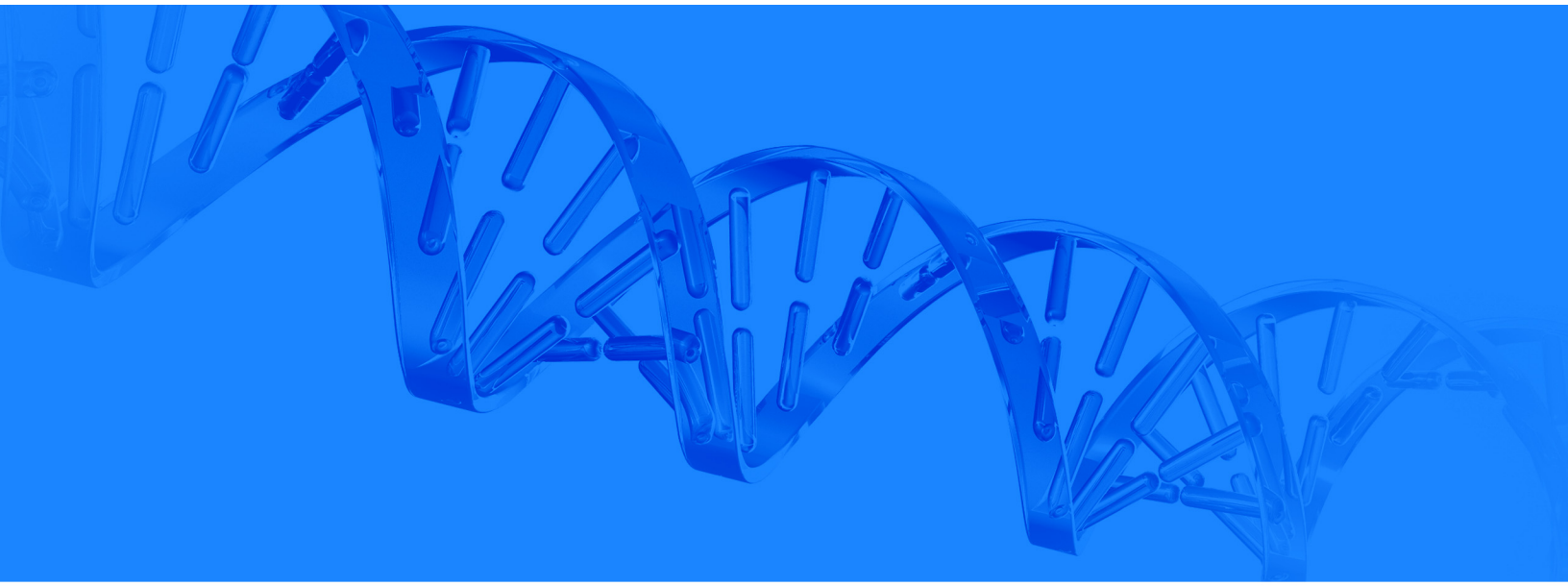
Applied Biosystems

TaqMan® Protein Expression Assay



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TRADEMARKS:

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Part Number 4405786 Rev. D
07/2010

Contents

Safety information

Note: For general safety information, see this Preface and Appendix E, "Safety" on page 33. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

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.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 35.

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

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

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

How to obtain 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, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

TaqMan® Protein Expression Assay Kit

Product overview

TaqMan® protein expression assays

The TaqMan® protein expression assay and reagent kits are used to perform relative quantitation of protein targets in mammalian cell culture samples. Several predesigned TaqMan protein expression assays are available for the detection of protein targets commonly associated with various applications of stem cell research. For a list of TaqMan protein expression assays currently available from Applied Biosystems, see "Available assays" on page 23.

About this protocol

The *TaqMan® Protein Expression Assay Protocol* provides step-by-step instructions for performing experiments using TaqMan® Protein Expression Assay Kit.

This protocol describes the:

- TaqMan Protein Expression Assay Kits and related kits and reagents.
- Equipment and materials that are required for performing the protein expression assay.
- Procedures and guidelines for performing an expression assay using the Protein Quantitation Kit.

Audience

This protocol is intended for novice and experienced laboratory personnel who perform experiments using the Protein Quantitation Kit.

Assumptions

This protocol assumes that you have a working knowledge of general techniques for handling cell cultures and harvesting cells for lysis.

Compatible real-time instruments

Applied Biosystems recommends using Protein Quantitation Kit with these instruments:

- StepOnePlus™ Real-Time PCR System
- 7500 Fast Real-Time PCR System
- 7900HT Fast Real-Time PCR System
- 7900HT Real-Time PCR System

How to order materials

For information on how to order Protein Quantitation Kit, Control Lysate kits, Core Reagents kits, and related products, see Appendix A, "Ordering Information" on page 23 or refer to the TaqMan® Protein Expression Assays product page at: **www.appliedbiosystems.com**.

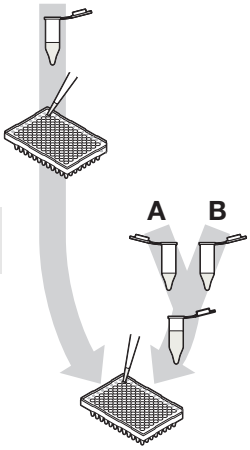
Workflow

Prepare the lysate dilutions

Prepare a serial dilution of the reference cell lysate
(Cell Resuspension Buffer)

↓

Prepare dilutions of the test cell lysate
(Cell Resuspension Buffer)



Perform the binding reactions

Prepare the Assay Probe solution
(Assay Probe A, Assay Probe B, Assay Probe Dilution Buffer)

↓

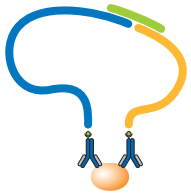
Plate the binding reactions, then incubate the plate

Perform the ligation reactions

Prepare the ligation solution
(DNA Ligase, Ligase Dilution Buffer, Ligation Reaction Buffer)

↓

Plate the ligation reactions, then incubate the plate



↓

Perform the protease stop reaction
(Protease, PBS)

Perform the real-time PCR reactions

Prepare the PCR master mix
(TaqMan® Protein Expression Fast Master Mix, Universal PCR Assay)

↓

Plate the reactions

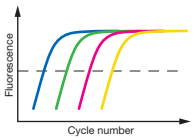
↓

Perform real-time PCR

Analyze the data

Export the analyzed data

↓



Prepare the lysate dilution plate

Set up the plate

The example plate setup shown in Figure 1 is for one reference and one test sample, with two-fold dilutions and four replicates of each dilution. Applied Biosystems recommends either two-fold or three-fold dilutions and three or four replicates. Your setup may be different. For suggested alternative plate layouts, see Appendix D, "Suggested Alternative Plate Layouts" on page 31.)

IMPORTANT! It is essential to include a no-protein control (NPC or zero-lysate input) for each pair of Assay Probes used.

When using cell lysates from the Protein Expression Lysate Control Kits (Raji or NTERA2), you can use Lysate Dilution Buffer instead of Cell Resuspension Buffer, at the same volumes and dilutions.

IMPORTANT! Follow the guidelines for transferring reagents as explained in "Pipetting guidelines" on page 27.

Prepare the dilutions

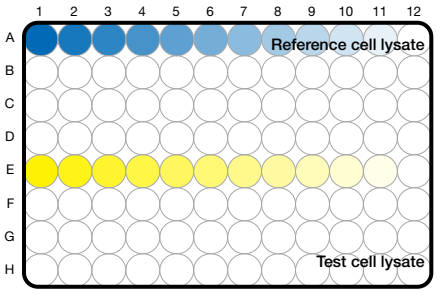
For the following hazard, see the complete safety alert descriptions in Appendix E, "Safety" on page 33.

WARNING! CHEMICAL HAZARDS. Cell Resuspension Buffer, Lysate Dilution Buffer, Lysate Control.

Applied Biosystems recommends that you use:

- Diluted cell lysates (starting at 500 cells/µL)
- The same concentration for each sample

IMPORTANT! Applied Biosystems strongly recommends that you prepare the dilutions on ice.



Lysate dilution plate

Figure 1 Lysate dilution plate setup

1. Put the following materials on ice to thaw:
   - Cell Resuspension Buffer from the sample preparation kit (When using cell lysates from the Protein Expression Lysate Control Kits [Raji or NTERA2], you can use Lysate Dilution Buffer instead of Cell Resuspension Buffer, at the same volumes and dilutions.)
   - Reference and test cell lysates prepared using the Protein Expression Sample Preparation Kit or Raji or NTER2 Control Lysates from the Protein Expression Lysate Control Kits.

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

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

   a. Pipette 12 µL of Cell Resuspension Buffer into wells A1 through A12.

   Note: When using cell lysates from the Protein Expression Lysate Control Kits [Raji or NTERA2], you can use Lysate Dilution Buffer instead of Cell Resuspension Buffer, at the same volumes and dilutions.

   b. Pipette 12 µL of reference cell lysate (500 cells/µL) into well A1. Pipette up and down several times to mix the sample.

   c. Continue to transfer 12 µL of 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 cell lysate).

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

   a. Pipette 12 µL of Cell Resuspension Buffer into wells E1 through E12.

   Note: When using cell lysates from the Protein Expression Lysate Control Kits [Raji or NTERA2], you can use Lysate Dilution Buffer instead of Cell Resuspension Buffer, at the same volumes and dilutions.

   b. Pipette 12 µL of test lysate (500 cells/µL) into well E1. Pipette up and down several times to mix the solution.

   c. Continue to transfer 12 µL of 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 cell lysate).

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

5. Put the plate on ice.

IMPORTANT! Do not store the lysate dilution plate overnight.

Perform the binding reaction

Guidelines for preparing the binding reaction

In addition to the guidelines for transferring reagents explained in "Pipetting guidelines" on page 27, follow the guidelines below when preparing the binding reactions:

Visually inspect the pipette tips to ensure consistent volume aspiration and delivery of reagents.

Volumetric variation generates significant variations in the threshold cycler (C_T) values calculated by the real-time PCR system.

Prepare the Assay Probe solution

For the following hazard, see the complete safety alert descriptions in Appendix E, "Safety" on page 33.

WARNING! CHEMICAL HAZARDS. Assay Probe Dilution Buffer (1×), Assay Probe A (20×), Assay Probe B (20×).

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

1. Put the following reagents on ice:
   - Assay Probe Dilution Buffer
   - Assay Probe A
   - Assay Probe B
2. Mix the reagents completely:
   a. When the Assay Probe Dilution Buffer is completely thawed, briefly vortex the tube to mix the solution.
   b. Touch-vortex the Assay Probe A and Assay Probe B tubes to mix the solutions.
3. Combine the reagents:
   a. Combine in a tube of appropriate volume:

Assay Probe solution	Volume (µL)‡
Assay Probe Dilution Buffer, 1×	216
Assay Probe A, 20×	12
Assay Probe B, 20×	12
Total Volume	**240**

‡ Includes excess volume to provide for any loss that occurs during reagent transfers.

   b. Briefly vortex the tube to mix the solution, then centrifuge the tube (for example, 700 × g for 30 seconds).

4. Put the Assay Probe solution on ice.

Perform the binding reaction

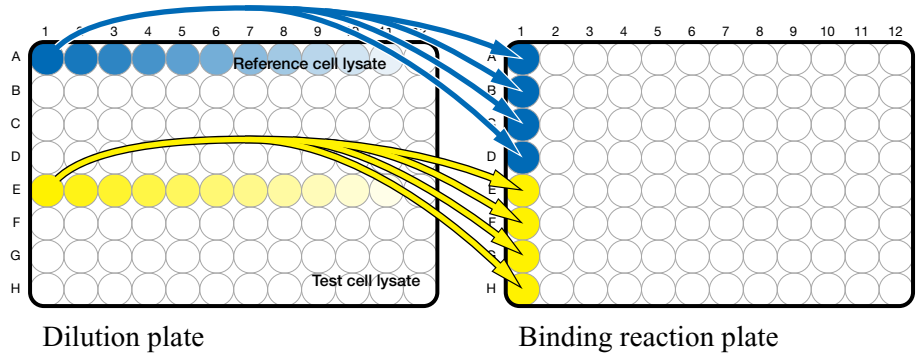


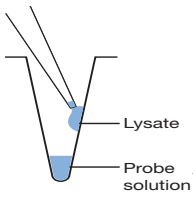
Figure 2 Binding reaction plate setup

1. Put a 96-well plate on ice.

   IMPORTANT! Applied Biosystems strongly recommends that you prepare the binding reactions while the plates are on ice.

2. Pipette 2 µL of the Assay Probe solution into each well of the binding reaction plate.

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



3. Add the reference cell lysate dilutions:
   a. Pipette 2 µL of the diluted reference cell lysate from wells 1 to 12 of row A in the lysate dilution plate into the corresponding wells of row A in the binding reaction plate, as shown in Figure 2.
   b. Repeat step a for rows B through D of the binding reaction plate.
4. Add the test cell lysate dilutions:
   a. Pipette 2 µL of the diluted test cell lysate from wells 1 to 12 of row E in the lysate dilution plate into the corresponding wells of row E in the binding reaction plate as shown in Figure 2.
   b. Repeat step a for rows F through H of the binding reaction plate.
5. Seal the binding reaction plate, using a MicroAmp® Clear Adhesive Film.
6. Briefly centrifuge the plate.

7. Put a MicroAmp® Optical Film Compression Pad on top of the sealed plate to form a tight seal and to prevent evaporation during incubation. Using a thermal cycler with a heated cover, incubate the sealed plate at 37 °C for 60 minutes.

Stage	Temp (°C)	Time (mm:ss)
Hold	37	60:00
Hold	4	∞

Note: Keep the binding reactions at 4 °C until you are ready to open the binding reaction plate. Applied Biosystems recommends that you proceed to the next step within 15 minutes.

Perform the ligation and protease reactions

Guidelines for the ligation and protease reactions

In addition to the guidelines for transferring reagents explained in "Pipetting guidelines" on page 27, follow the guidelines below when preparing the ligation and protease reactions:

- Visually inspect the pipette tips to ensure consistent volume aspiration and delivery of reagents.

  Volumetric variation generates significant variations in the threshold cycler (C_T) values calculated by the real-time PCR system.
- Use reservoirs and multichannel pipettors to aliquot the ligation and protease reagents.

Perform the ligation reaction

For the following hazard, see the complete safety alert descriptions in Appendix E, "Safety" on page 33.

WARNING! CHEMICAL HAZARDS. DNA Ligase (500×), Ligase Dilution Buffer, Ligation Reaction Buffer (20×), Phosphate Buffered Saline (PBS, 1×, pH7.4), Protease (100×), Universal PCR Assay Mix (20×).

1. Put the following materials on ice to thaw:
   - DNA Ligase, 500×
   - Ligase Dilution Buffer
   - Ligation Reaction Buffer, 20×
   - Phosphate Buffered Saline (PBS), 1×, pH 7.4 (for protease reactions)
   - Protease, 100×

   IMPORTANT! Put all reagents on ice when not in use. Avoid allowing the tubes to warm to room temperature.

2. Dilute the DNA Ligase with Ligase Dilution Buffer:

   IMPORTANT! Prepare fresh diluted ligase for each experiment.

   a. Gently flick the DNA Ligase tube several times to mix the solution, then briefly centrifuge the tube.

   b. Briefly vortex the Ligase Dilution Buffer to mix the solution, then briefly centrifuge the tube.

   c. Combine in a tube of appropriate volume:

Diluted ligase	Volume (µL)
DNA Ligase, 500×	2.0
Ligase Dilution Buffer	998
Total Volume	**1000**

d. Gently flick or touch vortex, then put the diluted ligase on ice.

3. Prepare the ligation solution:

   a. When the Ligation Reaction Buffer is thawed, briefly vortex the tube to mix the solution.

   b. Combine in a tube of appropriate volume:

Ligation solution	Volume (µL)‡	
	1 reaction	120 reactions (1 plate)
Ligation Reaction Buffer, 20×	5.0	600
H_2O, deionized	90.9	10,908
Diluted ligase (from step 2)	0.1	12
Total	**96**	**11,520**

‡ Includes excess volume to provide for any loss that occurs during reagent transfers.

   c. Invert the tube to mix the components.

4. Pipette 96 µL of the ligation solution into each binding reaction:

   a. Remove the binding reaction plate from the thermal cycler.

   b. Remove the MicroAmp® Clear Adhesive Film from the binding reaction plate, then put the plate on ice.

   c. Pipette 96 µL of the ligation solution into each well in the binding reaction plate. Pipette up and down once to mix.

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

6. Briefly centrifuge the ligation reaction plate.

7. Put a MicroAmp® Optical Film Compression Pad on top of the sealed ligation reaction plate.

8. Using a thermal cycler with a heated cover, incubate the resealed plate at 37 °C for 10 minutes.

Stage	Temp (°C)	Time (mm:ss)
Hold	37	10:00
Hold	4	∞

IMPORTANT! Keep the ligation reaction plate at 4 °C only long enough to prepare the protease reaction (no longer than 10 minutes), then perform the protease reaction immediately.

Perform the protease reaction

1. Verify that the Protease and the PBS are completely thawed, briefly vortex each tube to mix the solution, then briefly centrifuge each tube.
2. Combine in a tube of appropriate volume:

Diluted protease	Volume (µL)‡
Protease, 100×	4
Phosphate Buffered saline (PBS), 1×, pH 7.4	396
Total	**400**

‡ Includes excess volume to provide for any loss that occurs during reagent transfers.

IMPORTANT! Prepare the protease immediately before use.

3. Briefly vortex, then put the diluted protease solution on ice.
4. Pipette 2 µL of diluted protease solution into the wells of the ligation reaction plate to terminate the ligation reaction:
   a. Remove the reaction plate containing the ligation reactions from the thermal cycler.
   b. Remove the MicroAmp® Clear Adhesive Film from the ligation reaction plate, then put the plate on ice.
   c. Pipette 2 µL of the protease solution into each well on the ligation reaction plate.

   Note: No mixing is required. Protease activity diffuses throughout the sample during the 10-minute incubation.
5. Using a new MicroAmp Clear Adhesive Film, reseal the reaction plate.
6. Put a MicroAmp® Optical Film Compression Pad on top of the sealed reaction plate.

7. Using a thermal cycler with a heated cover, incubate the resealed plate at 37 °C for 10 minutes, then 95 °C for 5 minutes.

Stage	Temp (°C)	Time (mm:ss)	Description
Hold	37	10:00	Terminate ligation
Hold	95	5:00	Inactivate protease
Hold	4	∞	

You can store the protease-treated ligation products for ≤ 3 days at 4 °C, or ≤ 2 weeks at –20 °C.

Perform the real-time PCR

Guidelines for performing the real-time PCR

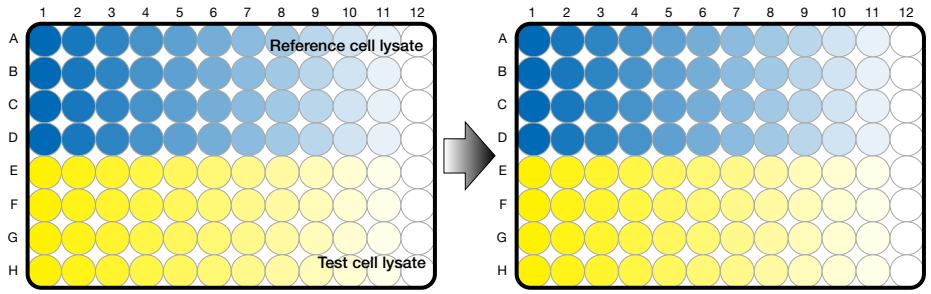
In addition to the guidelines for transferring reagents explained in "Pipetting guidelines" on page 27, follow the guidelines below when preparing the real-time PCR plate:

- Power on your Applied Biosystems real-time PCR instrument at least 30 minutes before loading the PCR reaction plate.
- (7900HT system users only) Confirm that the instrument door is in the closed position until just prior to loading the PCR reaction plate.

Prepare the real-time PCR plate

For the following hazard, see the complete safety alert descriptions in Appendix E, "Safety" on page 33.

WARNING! CHEMICAL HAZARD. Universal PCR Assay Mix (20×), TaqMan® Protein Expression Fast Master Mix (2×).



Protease-treated reaction plate

MicroAmp® Optical 96-Well Reaction Plate

Figure 3 PCR plate setup

1. Put the following materials on ice:
   - TaqMan® Protein Expression Fast Master Mix, 2×
   - Universal PCR Assay, 20×

   Put a MicroAmp® Optical 96-well Reaction Plate on ice. Use a Fast MicroAmp® Optical 96-well Reaction Plate if you use a 7900HT Fast, 7500 Fast, or StepOnePlus™ instrument.

2. Gently flick or touch vortex the TaqMan® Protein Expression Fast Master Mix and Universal PCR Assay, then briefly centrifuge the tubes.

3. In a microcentrifuge tube of appropriate volume, pipette the volumes of the PCR reagents shown:

Real-time PCR master mix	Volume (µL)‡	
	1 reaction	120 reactions (1 plate)
TaqMan® Protein Expression Fast Master Mix, 2×	10	1200
Universal PCR Assay, 20X	1	120
Total	**11**	**1320**

‡ Includes excess volume to provide for any loss that occurs during reagent transfers.

4. Briefly vortex, then centrifuge the master mix/assay solution.

5. Pipette 11 µL of the master mix/assay solution into each well of the Optical 96-well Reaction Plate.

6. Remove the reaction plate containing the protease-treated ligation product from the thermal cycler, then put the plate on ice.

7. Remove the MicroAmp® Clear Adhesive Film from the protease-treated reaction plate.

8. Pipette 9 µL of protease-treated ligation product into the corresponding wells of the Optical 96-well Reaction Plate. Pipette up and down once.

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

9. Seal the PCR plate, making sure to use a MicroAmp® Optical Adhesive Cover, not a standard adhesive cover.

10. Briefly centrifuge the plate.

    If you perform the run on a 7900HT Fast system with a 96-well Fast block and an automation accessory, put a MicroAmp® Snap-On Optical Film Compression Pad on top of the plate.

11. Load the MicroAmp® Optical 96-Well Reaction Plate into your real-time PCR instrument.

    (7900HT system users only) Confirm that the instrument door is in the closed position until just prior to loading the PCR reaction plate.

IMPORTANT! Run the real-time PCR reaction plate immediately after you complete the reaction setup.

Perform the real-time PCR reactions

1. Create a plate document/experiment for the run using the parameter values shown in Table 1. Table 2 provides details on the values listed in Table 1.
2. Load the plate into a StepOnePlus™, 7500 Fast, 7900HT Fast, or 7900HT Real-Time PCR System instrument.
3. Run the plate.

   For more instructions on how to create and run a plate document/experiment, refer to the documentation for your instrument (see "Related documentation" on page 43).

Table 1 Plate document/experiment setup information

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	Fast				Standard
Reaction plate	96-well Fast				96-well Standard
Sample volume	20 µL				20 µL
Detectors/ targets	Reporter: FAM™ dye Quencher: Non-fluorescent				Reporter: FAM™ dye Quencher: Non-fluorescent
Ramp speed/ mode	Fast				Standard

Table 2 Thermal cycling conditions

Run type	Reaction plate	Stage	Temp (°C)	Time (Enzyme activation, Denaturation, Annealing/Extension)
StepOnePlus™ system				
Fast	96-well Fast	Hold	95	20 seconds
		Cycle (40 cycles)	95	1 second
			60	20 seconds
7500 Fast system				
Fast	96-well Fast	Hold	95	20 seconds
		Cycle (40 cycles)	95	3 seconds
			60	30 seconds
7900HT Fast system				
Fast	96-well Fast	Hold	95	20 seconds
		Cycle (40 cycles)	95	1 second
			60	20 seconds

Run type	Reaction plate	Stage *(continued)*	Temp (°C)	Time (Enzyme activation, Denaturation, Annealing/Extension)
7900HT system				
Standard	96-well standard	Hold	95	2 minutes
		Cycle (40 cycles)	95	15 seconds
			60	1 minute

Analyze the real-time data

Analyze the data

To analyze the data from TaqMan® protein expression experiments:

1. View the amplification plots for the entire plate.
2. Analyze the plate run using a threshold setting of 0.2 with automatic baseline (for all systems).
3. Export the C_T data for comparative analysis.

   For information on exporting data, refer to your instrument protocol (See "Related documentation" on page 43.

For more information

For more information on relative quantitation:

1. Go to **www.appliedbiosystems.com**.
2. In the Home page of the Applied Biosystems web site, click **Store Log In** (or click **Log In**), then log in using your customer account information.
3. Select **Products ▸ Real-Time PCR ▸ Protein Expression Assays**.

For information on data analysis, see "Resources for data analysis" on page 44.

Troubleshooting

For detailed troubleshooting information for the TaqMan® protein expression experiments, refer to the *Real-Time PCR Systems Protein Expression Assays Chemistry Guide* (See "Related documentation" on page 43.)

Table 3 Troubleshooting

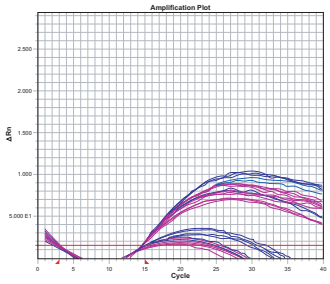
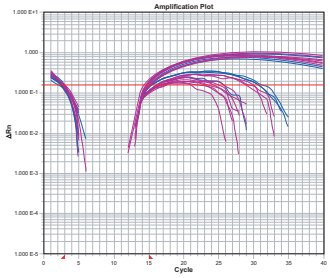
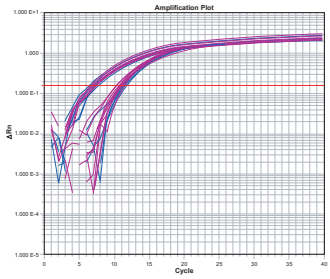
Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔR_n values Linear view:  Log view: 	The baseline was set improperly (some samples have C_T values lower than the baseline stop value)	Refer to your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, 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.

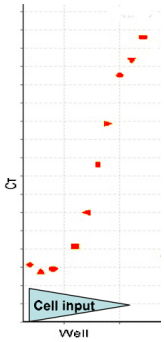
Table 3 Troubleshooting *(continued)*

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.
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	Check the volumes in each well. Check the seal of the optical adhesive cover 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.
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.

Table 3 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
High standard deviation of replicates (inconsistent data, 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.
	Threshold was set improperly	Use a threshold of 0.2.
	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 established PCR good laboratory practices.
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.
	Incomplete ligase inactivation	
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.
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
	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.	• Reset the lower value of the baseline range. • Use automatic baselining.
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	Check the seal of the optical adhesive cover for leaks, and check the volumes in each well.
	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.

Table 3 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
Higher cell input produces higher C_T than lower 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 Protein Expression Assays Chemistry Guide.*

Required materials and equipment

Available assays

Table 4 shows the TaqMan® Protein Expression Assay Kits currently available from Applied Biosystems. When you receive the Protein Quantitation Kits, Lysate Control Kits, or Core Reagents Kits, store the reagents as explained in the table. For a list of user-supplied materials, see "Other related materials" on page 25. To order new assays that become available, see "Ordering from the Applied Biosystems website" on page 26.

Table 4 TaqMan® Protein Expression Assay Kit

TaqMan® Protein Expression Assay Kits	Contents	Rxns	Storage conditions	Part Number
Human CSTB Kit (PN 4405465)	Assay Probe A, 20× (20 µL)	100	–15 to –25 °C	4405407 (hCSTB)
Human ICAM1 Kit (PN 4405471)				4405413 (hICAM1)
Human LIN28 Kit (PN 4405477)				4405419 (hLIN28)
Human NANOG Kit (PN 4405483)				4405425 (hNANOG)
Human OCT3/4 Kit (PN 4405489)				4405431 (hOCT3/4)
Human SOX2 Kit (PN 4405495)				4405437 (hSOX2)
	Assay Probe B, 20× (20 µL)			4405410 (hCSTB)
				4405416 (hICAM1)
				4405422 (hLIN28)
				4405428 (hNANOG)
				4405434 (hOCT3/4)
				4405440 (hSOX2)
	Assay Probe Dilution Buffer, 1× (0.5 mL)	100	–15 to –25 °C ‡	4405404

‡ The Assay Probe Dilution Buffer should be kept frozen for long-term storage. However, it may be stored at 2 to 8 °C for ≤1 month after initial use.

Reagents

In addition to ordering assay(s) for your TaqMan protein expression experiments, you should order the core reagents (Table 5) required for the experiment.

Table 5 Core Reagents Kit

Kit	Contents	Rxns	Storage conditions	Part Number
TaqMan® Protein Expression Core Reagents Kit with Master Mix (PN 4405501)	TaqMan® Protein Expression Core Reagents Base Kit (PN 4405460) • DNA Ligase, 500× (10 µL) • Ligase Dilution Buffer, 1× (2 × 1.5 mL) • Ligation Reaction Buffer, 20× (0.7 mL) • Phosphate Buffered Saline (PBS), pH 7.4, 1× (1 mL) • Protease, 100× (10 µL) • Universal PCR Assay, 20× (120 µL)	100	–15 to –25 °C	• 4405389 (DNA Ligase) • 4405392 (Ligase Dilution Buffer) • 4405395 (Ligation Reaction Buffer) • 4412427 (PBS) • 4405398 (Protease) • 4405401 (Universal PCR Assay)
	TaqMan® Protein Expression Fast Master Mix, 2× (1.2 mL) (PN (4400088)	100	2 to 8 °C‡	4400085

‡ Optionally, the TaqMan Protein Expression Fast Master Mix can be frozen at –15 to –25 °C for long-term storage, prior to initial use.

Control cell lysate kits

Table 6 shows the TaqMan Protein Expression Lysate Control Kits currently available from Applied Biosystems. These Control Kits are for general use. You can use them as positive controls for troubleshooting. See "Ordering from the Applied Biosystems website" on page 26 for ordering instructions.

Table 6 TaqMan® Protein Expression Lysate Control Kits

Kit	Contents	Rxns‡	Storage conditions	Part Number
Raji Kit; cells express ICAM1 and CTSB (PN 4405448) NTERA2 Kit; cells express LIN28, NANOG, OCT3/4, and SOX2 (PN 4405454)	Lysate Control, 500 cells/µL (50 µL)	100	< –50 °C	4405380 (Raji) 4405386 (NTERA2)
	Lysate Dilution Buffer (1 mL)	100	–15 to –25 °C§	4405383

‡ The number of reactions includes the reactions performed using the 2-fold dilution scheme outlined on page 5.
§ The Lysate Dilution Buffer may be stored with the Lysate Control at < –50 °C.

Additional materials and equipment required to perform TaqMan protein expression experiments are explained in "Related materials and equipment" on page 25.

Sample Preparation Kit

The Applied Biosystems Protein Expression Sample Preparation Kit (Table 7) can be used to prepare test samples for TaqMan protein expression assays.

Table 7 Protein Expression Sample Preparation Kit

Kit	Contents	Samples	Storage conditions	Part Number
Protein Expression Sample Preparation Kit (PN 4405443)		25	2 to 8 °C	
	Cell Lysis Reagent, 2× (3 × 1 mL)			4405374
	Cell Resuspension Buffer (25 mL)			4405377

Related materials and equipment

Reaction plates and accessories

Table 8 shows accessories available for Applied Biosystems thermal cyclers and real-time PCR systems.

Table 8 Reaction plates and accessories for Applied Biosystems thermal cyclers and real-time PCR systems

Instrument (Fast 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 (PN 4311971) • MicroAmp Optical 8-Cap Strips, 300 strips (PN 4323032)
7900HT Fast system, standard 96-well block	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • MicroAmp Optical Adhesive Film (PN 4311971) • MicroAmp Optical Film Compression Pad (PN 4312639) for use with one plate • MicroAmp Optical 8-Cap Strips, 300 strips (PN 4323032)
7900HT Fast system, Fast 96-well block	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp Optical Adhesive Film (PN 4311971) • MicroAmp Optical Film Compression Pad (PN 4312639) for use with one plate
StepOnePlus™ system	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp Optical Adhesive Film (PN 4311971)

Other related materials

Unless otherwise indicated, all related materials inTable 9 are available from major laboratory suppliers (MLS).

Table 9 Required user-supplied materials

Material	Source
Thermal cycler (recommended) or incubator (for the 37 °C and 95 °C incubation steps)	Applied Biosystems or MLS
Centrifuge (with plate adapter)	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
Pipette tips, aerosol-resistant	MLS
Pipettors (positive/air-displacement or multichannel)	MLS
Polypropylene tubes (various sizes)	MLS
Reaction plates, 96-well, compatible	Applied Biosystems
Vortexer	MLS
DNA*Zap*™ Solution	Applied Biosystems

Ordering from the Applied Biosystems website

To order theTaqMan® Protein Expression Assay Kits, Core Reagents Kit, and Control Cell Lysates:

1. Go to **www.appliedbiosystems.com**.
2. In the Home page of the Applied Biosystems web site, click **Store Log In** (or click **Log In**), then log in using your customer account information.
3. Select **Products ▸ Real-Time PCR ▸ Protein Expression Assays.**
4. In the Protein Expression Assays page, select the assay or reference cell lysate of interest to view details about the assay or reference cell lysate.
5. Click **Add to Basket**.
6. Order the assay as instructed by the web site.

Recommended laboratory practices and guidelines

IMPORTANT! Keep all tubes and plates on ice while transferring reagents.

PCR good 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.

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).

Pipetting guidelines

Precise volume delivery, especially during the binding step, is crucial for the performance and reproducibility of TaqMan® Protein Expression experiments. Follow the guidelines below when transferring reagents during the sample preparation:

- Use repeat or multi-channel pipettors to transfer reagents.
- Verify that all tips are properly seated prior to fluid transfer.
- Use pipets that are calibrated regularly.

- Pre-aliquot reagents into a separate 96-well reaction plate or reservoir for loading into the multi-channel pipettor. 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.
- Visually inspect the pipette tips to ensure consistent volume aspiration and delivery of reagents.

Chemistry overview

How TaqMan® protein expression assays work

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

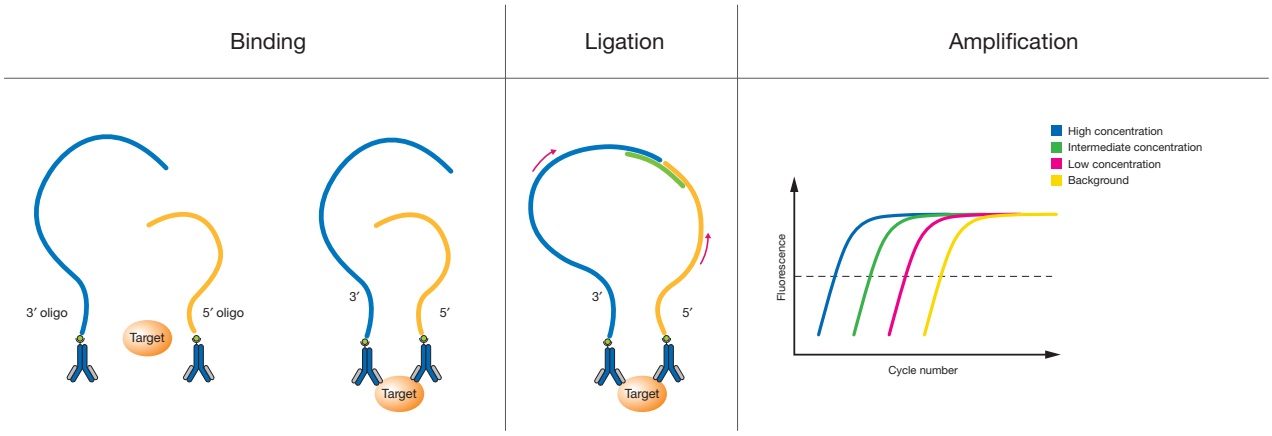


Figure 4 TaqMan® Protein Expression Assay process

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 Expression Fast Master Mix and TaqMan® Universal PCR Assay, which detects a target sequence that spans the junction of the ligated oligonucleotide chains.

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.

Analysis

For information on data analysis, refer to the TaqMan Protein Expression Assays product page at:**www.appliedbiosystems.com/products/protein expression**

For more information

For more information on TaqMan protein expression assays, refer to the following documentation.

- For details about the assay chemistry or data analysis, refer to the *Real-Time PCR Systems TaqMan® Protein Expression Assays Chemistry Guide* (See "Related documentation" on page 43).
- For more information on real-time PCR relative quantitation chemistry, refer to documents that shipped with your Applied Biosystems Real-Time PCR System:
  - **User guide** – Procedures for using and maintaining the instrument, including performing instrument calibrations
  - **Getting started guides for quantitation experiments** – Background information on real-time PCR, experiment examples, and guidelines for experiment design, setup, run, and analysis
  - **Chemistry guide or reagent guide** – Information on Applied Biosystems reagents and applications that are supported on your real-time PCR system

See "Related documentation" on page 43 for document titles and part numbers.

Suggested Alternative Plate Layouts

You can use a variety of plate layouts with the TaqMan® Protein Expression Assays. For example, you can use a single plate to perform relative quantitation of protein expression in more than one test cell lysate. Figures 5 through 7 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 5 Suggested plate layout with two test cell lysates

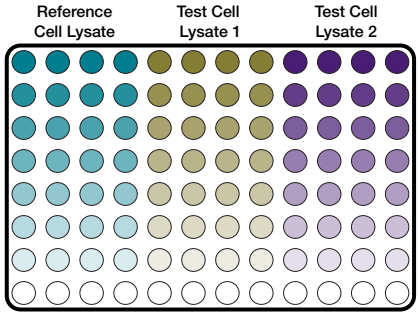


Figure 6 Suggested plate layout with three test cell lysates

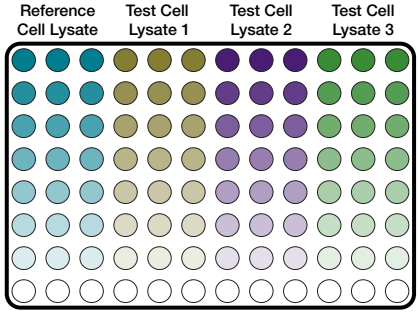
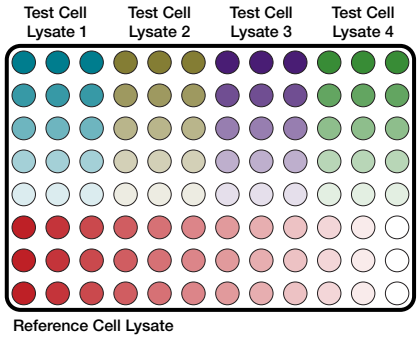


Figure 7 Suggested plate layout with four test cell lysates



Safety

This appendix covers:

General chemical safety

Chemical hazard warning

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

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter 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.

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 Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 35.)
- 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 MSDS.
- 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 MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

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

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

Obtaining MSDSs

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

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS 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 MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

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

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

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 Material Safety Data Sheets (MSDSs) 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 MSDS.
- 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 MSDS.
- 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 when you operate the instrument, 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 instrument 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* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- 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

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see"Safety alert words" on page v.

General alerts for all chemicals

CHEMICAL HAZARD. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

WARNING! 20× Assay Probe A may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves. This product contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 20× Assay Probe B may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves. This product contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 1× Assay Probe Dilution Buffer contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 1× Cell Resuspension Buffer contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 500× DNA Ligase may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves.

FIRST AID: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact, flush skin and eyes with plenty of water. Remove contaminated clothing and shoes. Get medical attention.

WARNING! 1× Ligase Dilution Buffer may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! 20× Ligation Reaction Buffer is harmful if swallowed or inhaled. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! 1× Lysate Control, NTERA2 causes eye irritation. May cause skin and respiratory tract irritation. Avoid breathing vapor. Avoid contact with eyes and skin. Keep container tightly closed. Use only with adequate ventilation. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves. This product contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 1× Lysate Control, Raji causes eye irritation. May cause skin and respiratory tract irritation. Avoid breathing vapor. Avoid contact with eyes and skin. Keep container tightly closed. Use only with adequate ventilation. Wash thoroughly after handling. Wear appropriate protective

eyewear, clothing, and gloves. This product contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 1× Lysate Dilution Buffer contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 1× Phosphate Buffered Saline, pH7.4. Read the MSDS.

WARNING! 100× Protease may cause eye, skin and respiratory tract irritation. May cause allergic respiratory reaction. Do not breathe vapor (or dust). Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! 2× TaqMan® Protein Expression Fast Master Mix causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Keep container tightly closed. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash thoroughly after handling. Wear appropriate protective eyewear, clothing, and gloves. This product contains sodium azide at a concentration that is considered not hazardous according to OSHA regulations. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! 20× Universal PCR Assay. Read the MSDS.

European Warnings

English	R36	Irritating to eyes.
English	R37	Irritating to respiratory system.
English	R38	Irritating to skin.
English	S24	Avoid contact with skin.
English	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
English	S37	Wear suitable gloves.
English	S60	This material and/or its container must be disposed of as hazardous waste.
German	R36	Reizt die Augen.
German	R37	Reizt die Atmungsorgane.
German	R38	Reizt die Haut.
German	S24	Berührung mit der Haut vermeiden.

German	S26	Bei Berührung mit den Augen sofort gründlich mit Wasser abspülen und Arzt konsultieren.
German	S37	Geeignete Schutzhandschuhe tragen.
German	S60	Dieser Stoff und/oder sein Behälter sind als gefährlicher Abfall zu entsorgen.
French	R36	Irritant pour les yeux.
French	R37	Irritant pour les voies respiratoires.
French	R38	Irritant pour la peau.
French	S24	Eviter le contact avec la peau.
French	S26	En cas de contact avec les yeux, laver immédiatement et abondamment avec de l'eau et consulter un spécialiste
French	S37	Porter des gants appropriés.
French	S60	Eliminer le produit et/ou son récipient comme un déchet dangereux.
Spanish	R36	Irrita los ojos.
Spanish	R37	Irrita las vías respiratorias.
Spanish	R38	Irrita la piel.
Spanish	S24	Evítese el contacto con la piel.
Spanish	S26	En caso de contacto con los ojos, lávense inmediata y abundantemente con agua y acúdase a un médico
Spanish	S37	Úsese guantes adecuados.
Spanish	S60	Elimínense el producto y o recipiente como residuos peligrosos.
Italian	R36	Irritante per gli occhi.
Italian	R37	Irritante per le vie respiratorie.
Italian	R38	Irritante per la pelle.
Italian	S24	Evitare il contatto con la pelle.
Italian	S26	In caso di contatto con gli occhi,lavare immediatamente e abbondantemente con acqua e consultare un medico
Italian	S37	Usare guanti adatti.
Italian	S60	Questo materiale e/o il suo contenitore devono essere smaltiti come rifiuti pericolosi
Dutch	R36	Irriterend voor de ogen.
Dutch	R37	Irriterend voor de ademhalingswegen.
Dutch	R38	Irriterend voor de huid.
Dutch	S24	Aanraking met de huid vermijden.

Dutch	S26	Bij aanraking met de ogen onmiddellijk met overvloedig water afspoelen en deskundig medisch advies inwinnen
Dutch	S37	Draag geschikte handschoenen.
Dutch	S60	Deze stof en/of de verpakking als gevaarlijk afval afvoeren.
Swedish	R36	Irriterar ögonen.
Swedish	R37	Irriterar andningsorganen.
Swedish	R38	Irriterar huden.
Swedish	S24	Undvik kontakt med huden.
Swedish	S26	Vid kontakt med ögonen spola genast med mycket vatten och kontakta läkare.
Swedish	S37	Använd lämpliga skyddskläder.
Swedish	S60	För bort detta ämne och/eller förpackningen som farligt avfall.
Finnish	R36	Ärsyttää silmiä.
Finnish	R37	Ärsyttää hengityselimiä.
Finnish	R38	Ärsyttää ihoa.
Finnish	S24	Varottava aineen joutumista iholle.
Finnish	S26	Aineen jouduttua silmiin huuhdeltava välittömästi runsaalla vedellä ja mentävä lääkäriin
Finnish	S37	Käytettävä sopivia suojakäsineitä.
Finnish	S60	Tämä aine ja/tai sen pakkaus hävitettävä vaarallisena jätteenä.
Danish	R36	Irriterer øjnene.
Danish	R37	Irriterer åndedrætsorganerne.
Danish	R38	Irriterer huden.
Danish	S24	Undgå kontakt med huden.
Danish	S26	Kommer stoffet i øjnene, skylles straks grundigt med vand og læge kontaktes..
Danish	S37	Brug egnede beskyttelseshandsker under arbejdet.
Danish	S60	Dette materiale og dets beholder skal bortskaffes som farligt affald.

Related documentation

Refer to these documents for information about using Applied Biosystems PCR systems as well as protein expression reagents and kits. For additional documentation, see "Resources for data analysis" on page 44 and "How to obtain support" on page 45.

Real-time PCR system	Document	PN/SN
All	*Real-Time PCR Systems Protein Expression Assays Chemistry Guide*	4405780
	TaqMan® Protein Expression Assay Protocol	4405786
	TaqMan® Protein Expression Assay Sample Preparation Kit Protocol	4405785
	TaqMan® Protein Expression Assay Quick Reference Card	4405784
	TaqMan® Protein Expression Assay Sample Preparation Kit Quick Reference Card	4405783
7900HT Fast system Fast or standard sample blocks	*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 User Bulletin: Performing Fast Gene Quantification	4369584
7300, 7500, and 7500 Fast systems	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide*	4347824
StepOne™ systems	*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

Portable document format (PDF) versions of this guide and the documents listed above are also available at **www.appliedbiosystems.com**.

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe Acrobat Reader software available from **www.adobe.com**.

Resources for data analysis

For more information about analyzing your data, refer to the user guide for your real-time PCR instrument.

Real-time PCR system	Document	Part number
7900HT Fast system	*Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantification Getting Started Guide*	4364014
	Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantification Using Comparative C_T Getting Started Guide	4364016
	Applied Biosystems 7900HT Fast Real-Time PCR System Quick Reference Card: Performing Fast Gene Quantification	4351892
	Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4352533
7300/7500/ 7500 Fast systems	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems Absolute Quantification Getting Started Guide*	4347825
	Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems Relative Quantification Getting Started Guide	4347824
	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™ systems	*Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments*	4376784
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments	4376785
All	*Real-Time PCR Systems Chemistry Guide: Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems*	4348358

How to obtain 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, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page 45.

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