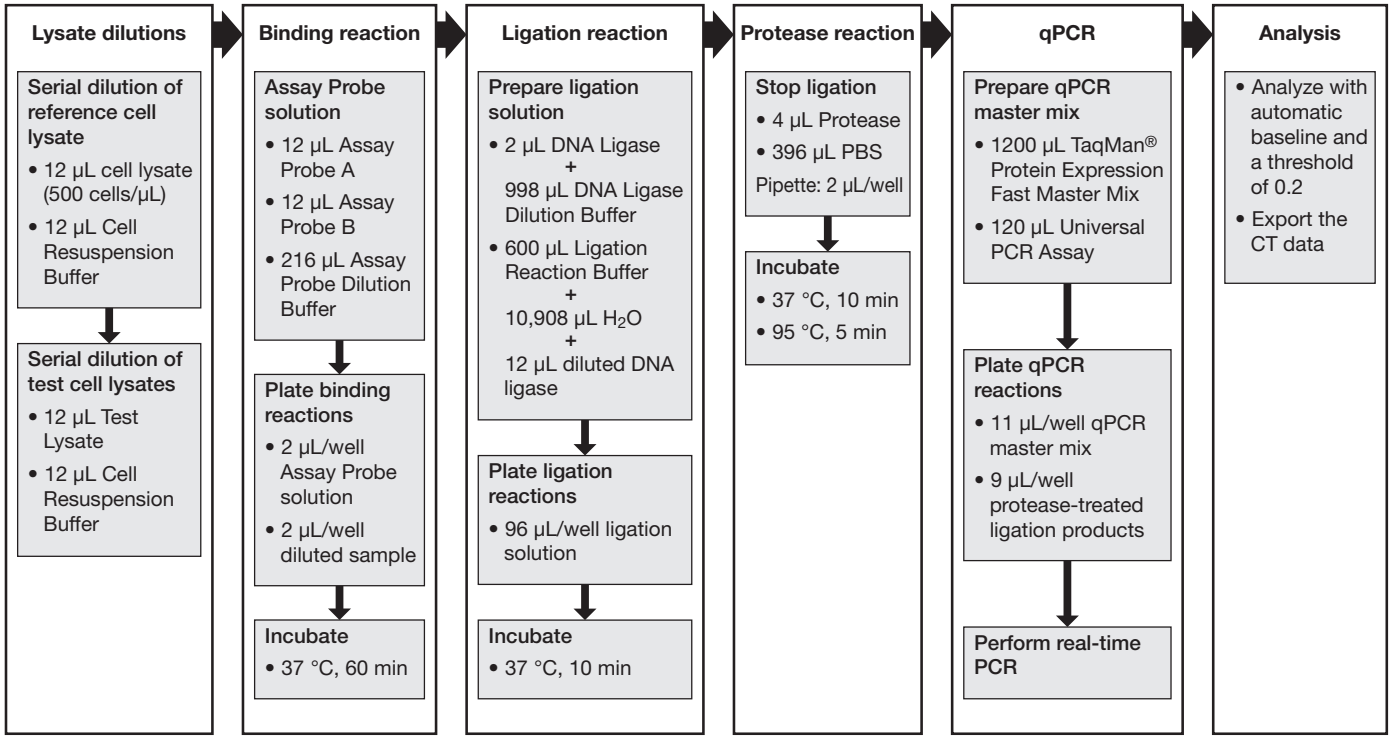
TaqMan® Protein Expression Assay

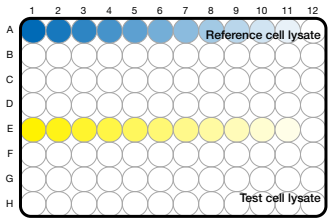
Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Protein Expression Assay Protocol* (PN 4405784). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow



Assay Steps

1 Prepare the lysate plate dilutions



This is a suggested plate layout. Refer to the *TaqMan® Protein Expression Assay Protocol* for other suggested plate layouts.

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

a. Put a 96-well plate on ice.

b. Thaw the lysates and buffer on ice.

c. In the 96-well reaction plate, pipette 12 µL of **Cell Resuspension Buffer** into wells A1 through A12.

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

d. Pipette 12 µL of reference cell lysate (500 cells/µL) into well A1. Pipette up and down several times.

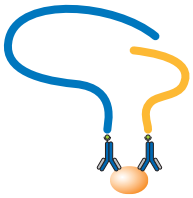
e. Transfer 12 µL of lysate from the previous dilution well into the next dilution well, pipetting up and down several times, for wells A2 through A11 (A12 contains no lysate).

f. Repeat step c through step e in row E, using your test cell lysate, then briefly centrifuge the plate to remove any bubbles.

g. Put the plate on ice.

IMPORTANT! Do not store the lysate dilution plate overnight.

2 Perform the binding reaction



a. Prepare the Assay Probe solution:

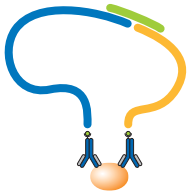
IMPORTANT! Prepare the Assay Probe solution no more than 20 min before performing the binding reaction.

1. Thaw, then briefly vortex the buffer. Thaw, then touch-vortex the Assay Probes. Combine:
   - 216 µL of **Assay Probe Dilution Buffer**
   - 12 µL of **Assay Probe A**
   - 12 µL of **Assay Probe B**
2. Briefly vortex, then briefly centrifuge the solution.
3. Put the solution on ice.

b. Combine the Assay Probe solution and diluted lysate samples in a reaction plate:

1. Put a 96-well plate on ice.
2. Pipette 2 µL of the Assay Probe solution into the bottom of each well of the reaction plate.
3. Pipette 2 µL of the diluted reference cell lysate from Row A of the lysate dilution plate onto the sides of corresponding wells in rows A-D of the reaction plate.
4. Pipette 2 µL of the diluted test cell lysate from Row E of the lysate dilution plate onto the sides of corresponding wells in rows E-H of the reaction plate.
5. Seal the plate. Briefly centrifuge the plate.
6. Put an Optical Film Compression Pad on top of the plate, then incubate the plate in a thermal cycler at 37 °C for 60 min. 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 min.

3 Perform the ligation reaction



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

a. Dilute the ligase:

IMPORTANT! Prepare fresh diluted ligase for each experiment.

1. Gently flick the tube of **DNA Ligase** several times, then briefly centrifuge.
2. Thaw, briefly vortex, then briefly centrifuge the **Ligase Dilution Buffer**. Combine:
   - 2 µL of DNA Ligase
   - 998 µL of Ligase Dilution Buffer
3. Briefly vortex and put the diluted ligase on ice.

b. Prepare the ligation solution:

1. Thaw, then briefly vortex the tube of **Ligation Reaction Buffer**. Combine:
   - 600 µL of Ligation Reaction Buffer
   - 10,908 µL of H_2O, deionized
   - 12 µL of diluted ligase

   Invert the tube to mix.
2. Remove the reaction plate from the thermal cycler, then put the plate on ice.
3. Pipette 96 µL of the ligation solution into each well of the reaction plate. Pipette up and down once. Reseal the plate, using a new adhesive film. Briefly centrifuge the plate.
4. Put an Optical Film Compression Pad on the plate. Incubate the plate at 37 °C for 10 min. Keep the plate at 4 °C only long enough to prepare the protease (≤ 10 min).

4 Perform the protease reaction

a. Thaw, briefly vortex, then briefly centrifuge the tubes of **Protease** and PBS. Combine:
   - 4 µL of Protease
   - 396 µL of **Phosphate Buffered Saline (PBS)**

IMPORTANT! Prepare the protease immediately before use.

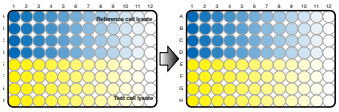
b. Briefly vortex and put the diluted protease solution on ice.

c. Remove the ligation reaction plate from the thermal cycler, then pipette 2 µL of diluted protease solution into each well.

d. Seal the plate with a new film.

e. Put a MicroAmp® Optical Film Compression Pad on the plate. Incubate the plate at 37 °C for 10 min, then at 95 °C for 5 min. After incubation, you can store the plate ≤ 3 days at 4 °C or ≤ 2 weeks at -20 °C.

5 Prepare the real-time PCR plate



a. Gently flick or touch-vortex the **TaqMan® Protein Expression Fast Master Mix** and the **Universal PCR Assay**, then briefly centrifuge the tubes. Combine:
   - 1200 µL of TaqMan® Protein Expression Fast Master Mix
   - 120 µL of Universal PCR Assay

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

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

c. Pipette 11 µL of the master mix/assay mixture into each well of the Optical Reaction Plate.

d. Remove the protease treated reaction plate from the thermal cycler. Put the plate on ice.

e. Pipette 9 µL of protease-treated ligation product into the corresponding wells of the Optical Reaction Plate. Pipette up and down once. Seal the plate, then briefly centrifuge the plate.

Note: Seal the plate with a MicroAmp® Optical Adhesive Cover, not a standard adhesive cover. If you use a 7900HT Fast instrument with a 96-well Fast block and automation accessory, put a Snap-on Optical Film Compression Pad on the plate.

f. Load the sealed Optical Reaction Plate into your real-time PCR instrument.

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

6 Perform the real-time PCR reactions

a. Create a plate document/experiment for the run, using the plate document and experiment setup information shown in Table 1 on page 4.

b. Use the thermal cycling conditions listed in Table 2 on page 4.

Note: Fast cycling conditions are recommended.

c. Run the plate.

7 Analyze the data

To analyze the data from TaqMan protein expression experiments:

a. View the amplification plots for the entire plate.

b. Analyze the plate run using a threshold setting of 0.2 with automatic baseline (for all systems).

c. Export the C_T data for comparative analysis.

   For information on exporting data, refer to your instrument protocol.

For information on relative quantitation:

a. Go to **www.appliedbiosystems.com**

b. In the Home page of the Life Technologies Corporation web site, click **Store Log In** (or click **Log In**), then log in using your customer account information.

c. Select **Products** ▸ **Protein Expression** ▸ **TaqMan® Protein Expression Assays**.

For information on data analysis, refer to the *TaqMan® Protein Expression Assay Protocol.*

Table 1 Run setup

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 sec
		Cycle (40 cycles)	95	1 sec
			60	20 sec
7500 Fast system				
Fast	96-well Fast	Hold	95	20 sec
		Cycle (40 cycles)	95	3 sec
			60	30 sec
7900HT Fast system				
Fast	96-well Fast	Hold	95	20 sec
		Cycle (40 cycles)	95	1 sec
			60	20 sec
7900HT system				
Standard	96-well standard	Hold	95	2 min
		Cycle (40 cycles)	95	15 sec
			60	1 min

10/2009