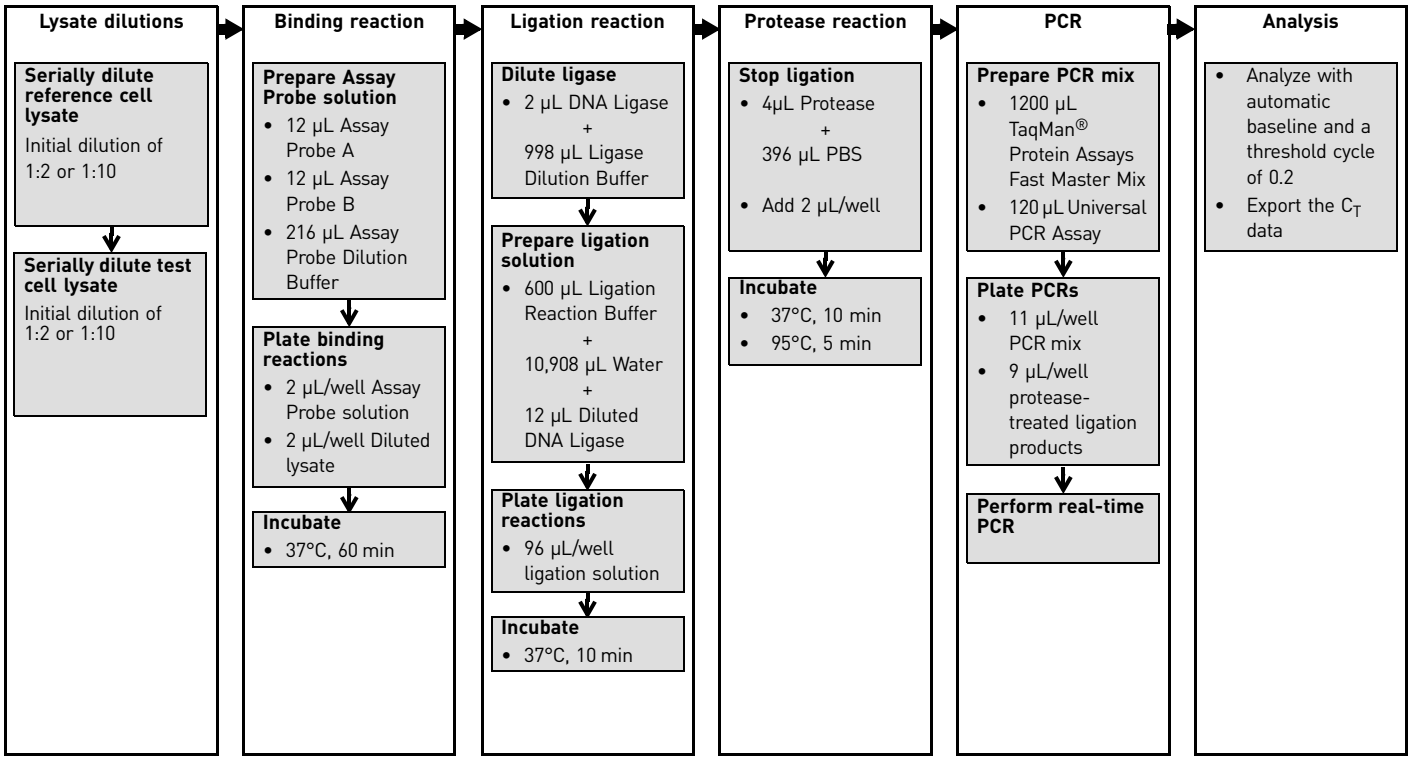
TaqMan® Protein Assays

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Protein Assays Sample Prep and Assays Protocol* (Part no. 4449283). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow

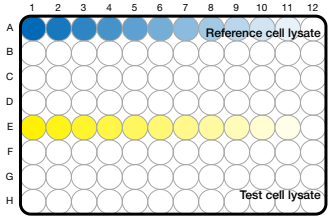


Prepare dilutions of each cell lysate

1 Determine the starting concentrations and minimum lysate dilutions

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 (Refer to the Protocol for a list of kits.)		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

2 Prepare the cell lysate dilutions



IMPORTANT! Prepare the cell lysate dilutions on ice. The diluted lysates must be used on the day of preparation. Do not store the diluted lysates overnight.

a. Thaw the appropriate buffer and lysates on ice, then place a 96-well reaction plate on ice.

b. Prepare 2-fold serial dilutions of the reference and test cell lysates:

Initial dilution	Step	Action	Reaction plate well number	
			Reference cell lysate	Test cell lysate
1:2	1	Add 12 µL of Cell Resuspension Buffer (or Lysate Dilution Buffer).†	A1 through A12	E1 through E12
	2	Add 12 µL of cell lysate. Pipet up and down several times to mix the sample.	A1	E1
	3	Continue to transfer 12 µL of cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times.	A11 is the last well; A12 contains no lysate	E11 is the last well; E12 contains no lysate
1:10	1	Add 18 µL of Lysate Dilution Buffer.†	A1	E1
	2	Add 12 µL of Lysate Dilution Buffer.	A2 through A12	E2 through E12
	3	Add 2 µL of cell lysate. Pipet up and down several times to mix the sample.	A1	E1
	4	Continue to transfer 12 µL of cell lysate from the previous dilution well to the next dilution well, pipetting up and down several times.	A11 is the last well; A12 contains no lysate	E11 is the last well; E12 contains no lysate

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

c. Briefly centrifuge the plate, then place the plate back on ice.

Perform the binding reaction

1 Prepare the Assay Probe solution

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

a. Place the following reagents on ice: Assay Probe Dilution Buffer, Assay Probe A, Assay Probe B.

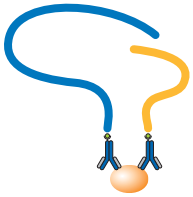
b. When thawed, gently mix the Assay Probe Dilution Buffer. Gently mix the Assay Probe A and Assay Probe B solutions.

c. Combine the components listed below *in the order indicated*. Mix gently, then centrifuge the tube. Place on ice.

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

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

2 Prepare and incubate the binding reaction plate



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

IMPORTANT! Keep the reaction plate on ice while you add the reaction components.

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

c. 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. Repeat for rows B through D of the binding reaction plate.

d. 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. Repeat for rows F through H of the binding reaction plate.

e. Seal the binding reaction plate with a MicroAmp® Clear Adhesive Film, then briefly centrifuge the plate.

f. Incubate the sealed reaction plate using the thermal-cycling conditions below.

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.

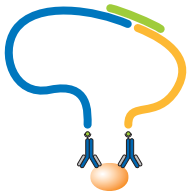
Stage	Temperature (°C)	Time
HOLD	37°C	60 minutes
HOLD	4°C	∞

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 4 within 15 minutes.

Perform the ligation and protease reactions

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

1 Perform the ligation reaction



a. Thaw or place the following components on ice: 500X DNA Ligase, 1X Ligase Dilution Buffer, 20X Ligation Reaction Buffer, 1X PBS (pH 7.4), 100X Protease.

b. Dilute the DNA Ligase: Combine the components listed below. Mix gently, then place on ice.

IMPORTANT! Prepare fresh diluted ligase for each experiment.

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

c. Prepare the ligation solution: Combine the components listed below. Invert the tube to mix, then place on ice.

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

d. Remove the binding reaction plate from the thermal cycler, remove the MicroAmp® Clear Adhesive Film, then place the plate on ice.

e. Add 96 µL of the ligation solution to each well in the binding reaction plate. Pipet up and down once to mix.

f. Reseal the ligation reaction plate with a new adhesive film, then briefly centrifuge the plate.

g. Incubate the sealed reaction plate using the thermal-cycling conditions below.

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°C	10 minutes	Default
Cooling	1	4°C	Up to 10 minutes	Default

You can omit the protease step if you proceed *immediately* to "Perform the real-time PCR" on page 6. Otherwise, continue with "Perform the protease reaction" on page 5.

2 Perform the protease reaction

a. Dilute the protease:

1. Briefly vortex the protease to mix the solution.
2. Combine the components listed below. Mix gently, then briefly centrifuge to spin the liquid to the tube bottom. Place on ice.

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

b. Remove the ligation reaction plate from the thermal cycler, remove the MicroAmp Clear Adhesive Film, 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.

d. Reseal the reaction plate with a new adhesive film.

e. Incubate the sealed reaction plate using the thermal-cycling conditions below.

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°C	10 minutes	Default
Inactivate protease	1	95°C	5 minutes	Default
HOLD	1	4°C	Hold	Default

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

Proceed to "Perform the real-time PCR" on page 6 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

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

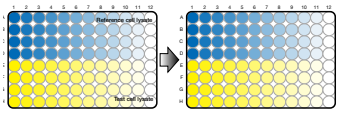
1 Prepare the PCR mix

a. Thaw the Universal PCR Assay on ice.

b. Combine the components listed below. Mix gently, then briefly centrifuge to spin the liquid to the tube bottom. Place on ice.

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

2 Prepare the PCR plate



a. Place a PCR plate on ice, then add 11 µL of the PCR mix to each well.

b. Remove the MicroAmp Clear Adhesive Film from the protease reaction plate, then place the plate on ice.

c. 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.

d. Seal the PCR plate with a MicroAmp® Optical Adhesive Film, then briefly centrifuge the plate. For the 7900HT/7900HT Fast system with a 96-Well Block Module and automation accessory, place a MicroAmp® Snap-On Optical Film Compression Pad on top of the plate.

IMPORTANT! Proceed *immediately* to "Run the PCR plate" on page 7.

3 **Run the PCR plate**

In your real-time PCR system software, create a plate document/experiment for the run, using the setup information below. Load the PCR plate into your real-time PCR instrument, then start the run.

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				

Analyze the data

Method	Procedure
Using the real-time PCR system software	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. 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	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.)

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