applied biosystems

ProteinSEQ™ CHO HCP Quantification Kit

USER GUIDE

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Revision	Date	Description
C.0	8 February 2019	Update the Manufacturer of Record address.
B.0	20 April 2018	Update template and legal information.
A.0	08 Dec 2014	New document.

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Contents

Product information

Product description

Use the ProteinSEQ™ CHO Host Cell Protein Quantification Kit to quantify the Chinese Hamster Ovary (CHO) Host Cell Proteins (HCPs) present in your bioprocess sample(s). TheProteinSEQ™ CHO HCP workflow consists of:

- Plate preparation
- A semi-automated sample processing run on the Pharma KingFisher™ Flex-96 Magnetic Particle Processor or the MagMAX™ Express-96 Magnetic Particle Processor
- A qPCR run on an Applied Biosystems™ 7500 Fast Real-Time PCR System (or equivalent system)
- Data analysis using AccuSEQ™ Real-Time PCR software or equivalent curve fitting software

Contents

Table 1 ProteinSEQ™ CHO HCP Quantification Kit (Cat. No. A27601)

Contents	Amount	Storage
Box (Cat. No. A25500)		
CHO HCP Standard (15.6 µg/mL)	0.25 mL	–25°C to –15°C Store at 2–8°C after thawing.[1]
CHO HCP 5′ Probe	0.35 mL	–25°C to –15°C
CHO HCP 3′ Probe	0.35 mL	
ProteinSEQ™ Ligation and Assay Mix	0.6 mL	
ProteinSEQ™ Ligase	55 µL	
Box (Cat. No. A25499)		
Fast Master Mix, 2X	6 mL	2–8°C
Wash Buffer	100 mL	
CHO HCP Capture Beads	5.5 mL	

Contents	Amount	Storage
ProteinSEQ™ Elution Buffer	7 mL	2–8°C
CHO HCP ProteinSEQ™ Diluent	50 mL	

[1] After thawing, do not re-freeze. Store at 2–8°C for up to 1 month.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**.

MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR System	4365464 (with notebook) 4365464 (with tower)
Benchtop microcentrifuge	MLS
Plate centrifuge	MLS
Benchtop vortexer	MLS
Software	
AccuSEQ™ Real-Time PCR software	4443420
Microsoft™ Excel™ software	microsoft.com
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
25-mL reagent reservoir	VistaLab Technologies™ 3054-1002 or equivalent
15-mL conical tube	AM12500 or equivalent
Pipettors • P20, P200, and P1000 single-channel pipettors • P200 and P1000 multichannel pipettors, 8- or 12-channel	MLS
qPCR plates • MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL[1] *or* • MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL[2]	• 4346906 *or* • 4306737

Item	Source
MicroAmp™ Optical Adhesive Film	4360954
Nonstick, RNase-free Microfuge Tubes, 1.5 mL	AM12450

[1] For use with thermal cycler FAST sample blocks.
[2] For use with thermal cycler standard sample blocks.

Table 2 Magnetic particle processor

Item	Source
KingFisher™ Flex-96 instrument and accessories	
Pharma KingFisher™ Flex-96 Magnetic Particle Processor	A31508
Pharma KingFisher™ Flex Magnetic Head for PCR Plate	A31544
Pharma MagMAX™ Express-96 Tip Combs for PCR Head	4472784
Additional plates • PCR Plate, low profile, skirted • KingFisher™ Flex 96 Standard Plates	 • AB-0800 • A31541
MagMAX™ Express-96 instrument and accessories	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	No longer available
MagMAX™ Express-96 PCR Well Magnetic Head	4472991
Pharma MagMAX™ Express-96 Tip Combs for PCR Head	4472784
Additional plates • PCR Plate, low profile, skirted • MagMAX™ Express-96 Standard Plates	 • AB-0800 • A31541

Methods

Workflow

Prepare serial dilutions of the CHO HCP standard (page 10)

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Prepare diluted samples (page 11)

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Prepare the plates for the run (page 12)

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Run the plates in the magnetic particle processor (page 14)

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Run the qPCR reaction (page 17)

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Perform data analysis (page 24)

Important procedural guidelines

- **IMPORTANT!** The magnetic particle processor's Magnetic Head is very fragile. The magnetic rods are easily bent or broken. Handle with care.
- Use serially diluted standards when performing spiking studies. See Appendix B, "Design guidelines for ProteinSEQ™ System HCP spike experiments" for spiking guidelines.
- Run all reactions in triplicate.
- We recommend digital multi-channel pipettors for transfers into the magnetic particle processor plates.
- Working solutions and plates can be kept at room temperature during assay setup.

Important serial dilution guidelines

- Prepare serial dilutions in 1.5-mL non-stick RNase-free microfuge tubes (Cat. No. AM12450 or equivalent). Or, if your standard dilution volume is ≤300 µL, you can prepare the serial dilutions in a MagMAX™ Express-96 Standard Plate (Cat. No. A31541 or equivalent polypropylene 96-well plate).
- Use a new pipette tip for each transfer.
- Pipet gently to minimize foaming and/or bubble formation.

- It is critical to mix standards during serial dilution. After each transfer,
  - **If preparing serial dilutions in microfuge tubes** — Invert the tube several times to mix.
  - **If preparing serial dilutions in a 96-well plate** — Gently pipet up and down 5–8 times to increase mixing efficiency.
- Prepare the standards at room temperature.

Important sample dilution guidelines

- Prepare sample dilutions in 1.5-mL non-stick RNase-free microfuge tubes, or in the dilution plate containing the CHO HCP standard serial dilutions.
- The numbers in the instructions are for preparing a 4X sample dilution. The required dilution depends on the process step that the samples came from. An early DSP sample will have to be more highly diluted. A sample from a later DSP step, or BDS, may not need to be diluted.
  - Dilute samples as needed to obtain final salt concentration in the reaction well <50 mM, and pH in the 6–9 range.
  - For samples with low CHO HCP concentrations, evaluate assay performance with lower dilutions to increase sensitivity. For samples with matrix interference, evaluate assay performance with higher dilutions. Generate a two- to five-fold dilution series to analyze dilution linearity in the sample matrix.

Before first use of the kit

- Contact your local sales or service representative to prepare your magnetic particle processor for use with ProteinSEQ™ assays and to obtain the following items:
  - The ProteinSEQ™ CHO HCP script for the magnetic particle processor (upload before you perform a ProteinSEQ™ assay for the first time)
  - The appropriate PCR Plate Adaptor (Fast or Standard)
  - If you are using GraphPad® software for data analysis, the CHO HCP Master Template
- Ask your local representative if your magnetic particle processor supports plate hold-downs. If supported, your local representative should install the plate hold-downs before you perform a ProteinSEQ™ assay for the first time.

Before each use of the kit

- The day before performing a ProteinSEQ™ assay: If the CHO HCP Standard is stored at –20°C, thaw the CHO HCP Standard in a 4°C refrigerator.

  Note: After thawing, store the CHO HCP Standard stock at 4°C for up to 1 month. Do not re-freeze.

- Before preparing the plates for each assay, clean the pipettors, plate racks and the microcentrifuge (if using tubes for standard dilution) to avoid cross-contamination.

Prepare serial dilutions of the CHO HCP standard

See "Important serial dilution guidelines" on page 8.

1. Label eight 1.5-mL microfuge tubes (see column 1 of Table 3) or a MagMAX™ Express-96 Standard Plate.
2. Dispense 160 µL CHO HCP ProteinSEQ™ Diluent to each of the eight tubes or plate wells (see Figure 1 on page 10).
3. Add 40 µL CHO HCP Standard (15.6 µg/mL) to the SD1 tube (or A1 plate well). Mix well. Do not vortex.
4. Use a new pipette tip to transfer 40 µL from SD1 to SD2 (or A1 to B1), then mix well. Do not vortex.
5. Repeat the procedure in step 4 to transfer the remaining dilutions (see the following table, column 3, and Figure 2 on page 11).

Table 3 Prepare serial dilutions.

Tube label	CHO HPC ProteinSEQ™ diluent	Dilution transfer	CHO HCP concentration
SD1	160 µL	40 µL of 15.6 µL/mL CHO HCP Standard	3125 ng/mL
SD2	160 µL	40 µL from SD1	625 ng/mL
SD3	160 µL	40 µL from SD2	125 ng/mL
SD4	160 µL	40 µL from SD3	25 ng/mL
SD5	160 µL	40 µL from SD4	5 ng/mL
SD6	160 µL	40 µL from SD5	1 ng/mL
SD7	160 µL	40 µL from SD6	0.2 ng/mL
NPC	160 µL	0	0

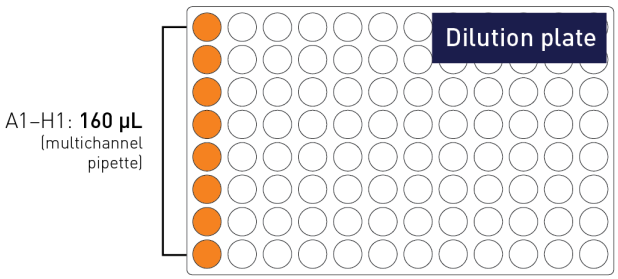


Figure 1 Dispense CHO HCP ProteinSEQ™ diluent (shown in plate format)

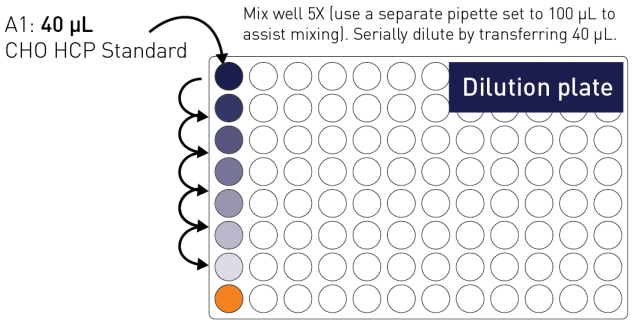


Figure 2 Serially dilute the CHO HCP standard from SD1 to SD7 (tubes) or A1 to G1 (shown in plate format)

Prepare diluted samples

See "Important sample dilution guidelines" on page 9.

Combine sample with CHO HCP ProteinSEQ™ Diluent. For example, combine 40-µL sample with 120 µL CHO HPC ProteinSEQ™ Diluent to prepare 160 µL of a 4X dilution.

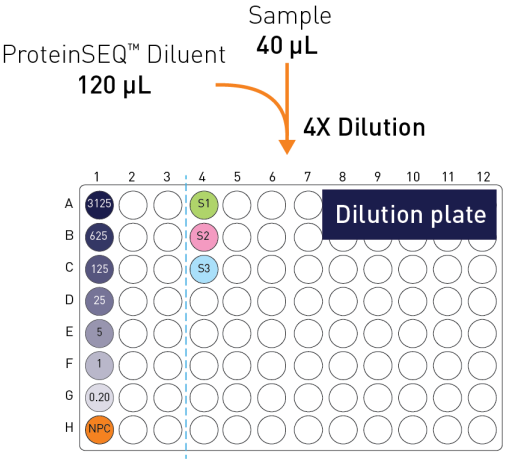


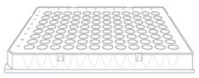
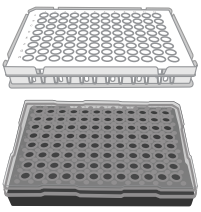
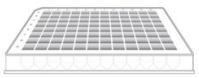
Figure 3 Dilute samples (example 4X dilution, shown in plate)

Prepare the plates for the run

Note: When preparing wash, capture, qPCR, and probes plates, dispense at bottom of wells to prevent bubble formation (bubbles prevent effective mixing during the magnetic particle processor run). If bubbles form, quick-spin the plate at 560 × *g* (~2,000 rpm) in a plate centrifuge.

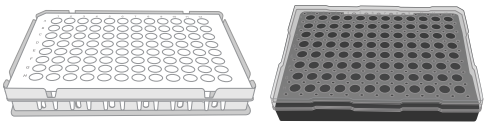
Label the plates

1. Label eight plates:

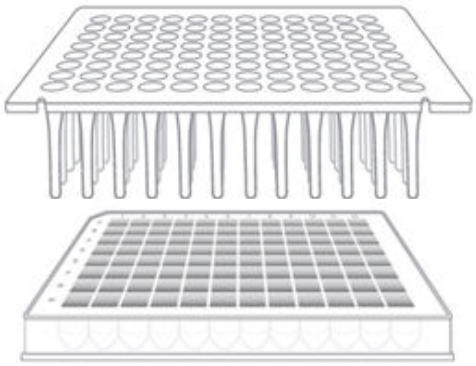
No. of plates	Plate type		Cat. No.	Label(s)
6	PCR Plate, 96-well, low profile, skirted		AB-0800	Capture Probes Wash 1 Wash 2 Wash 3 Wash 4
1	MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL)[1] *or* MicroAmp™ Optical 96-Well Reaction Plate with Barcode (0.2 mL)[2]		4346906 *or* 4306737	qPCR
1	MagMAX™ Express-96 Standard Plate (200 µL)		A31541[3]	Comb

[1] For use with thermal cycler FAST sample blocks; shown in Fast PCR Plate Adaptor.
[2] For use with thermal cycler standard sample blocks; shown in Standard PCR Plate Adaptor.
[3] Or equivalent polypropylene plate.

2. Insert the plate labeled "qPCR" into the appropriate PCR Plate Adaptor [Fast (on left) or Standard (on right); request from your local sales or service representative].

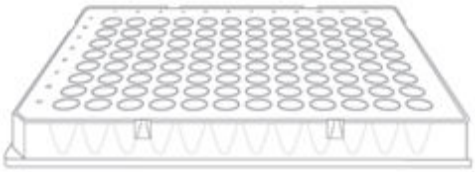


3. Place a MagMAX™ Express PCR Head Tip Comb (Cat. No. 4472784) in the plate labeled "Comb".



Prepare wash plates

1. Pour approximately 30 mL of Wash Buffer into a fresh reagent reservoir.
2. Dispense 100 µL of Wash Buffer into each well of the 4 wash plates with a multi-channel pipette.



Prepare qPCR plate

Dispense ProteinSEQ™ Elution Buffer into each well of the qPCR plate:

- Fast PCR plate—15 µL per well
- Standard PCR plate—25 µL per well

Prepare probes plate

1. Add the assay probe reagents to a 15-mL tube in the order shown in the table. Scale the volumes as needed for the number of reactions, including recommended overages. Vortex for 3 seconds at medium speed, then keep the 15-mL tube on ice.

Reagent	Cap color	Volume[1]		
		1 rxn	48 rxn	96 rxn
CHO HCP ProteinSEQ™ Diluent	Clear	59.4 µL	2,850 µL	5,700 µL
CHO HCP 5′ Probe	Grey	1.6 µL	75 µL	150 µL
CHO HCP 3′ Probe	Yellow	1.6 µL	75 µL	150 µL
Total		**62.5 µL**	**3,000 µL**	**6,000 µL**

[1] Includes 25% overage.

2. Invert the assay probe mix tube several times to mix, transfer to a reagent reservoir, then dispense 50-µL of assay probe mix into each well of the Probes plate with a multi-channel pipette.

Prepare capture plate

1. Vortex the CHO HCP Capture Beads for 3 seconds at medium speed, then pour into a 25-mL reagent reservoir.
2. Immediately dispense 20 µL of CHO HCP Capture Beads into each well of the Capture plate.

   Note: The remaining Capture Beads can be returned to the CHO HCP Capture Beads bottle for future use.

3. Transfer 30 µL of each standard and sample to the capture plate in triplicate. The final volume in the capture plate is 50 µL per well.

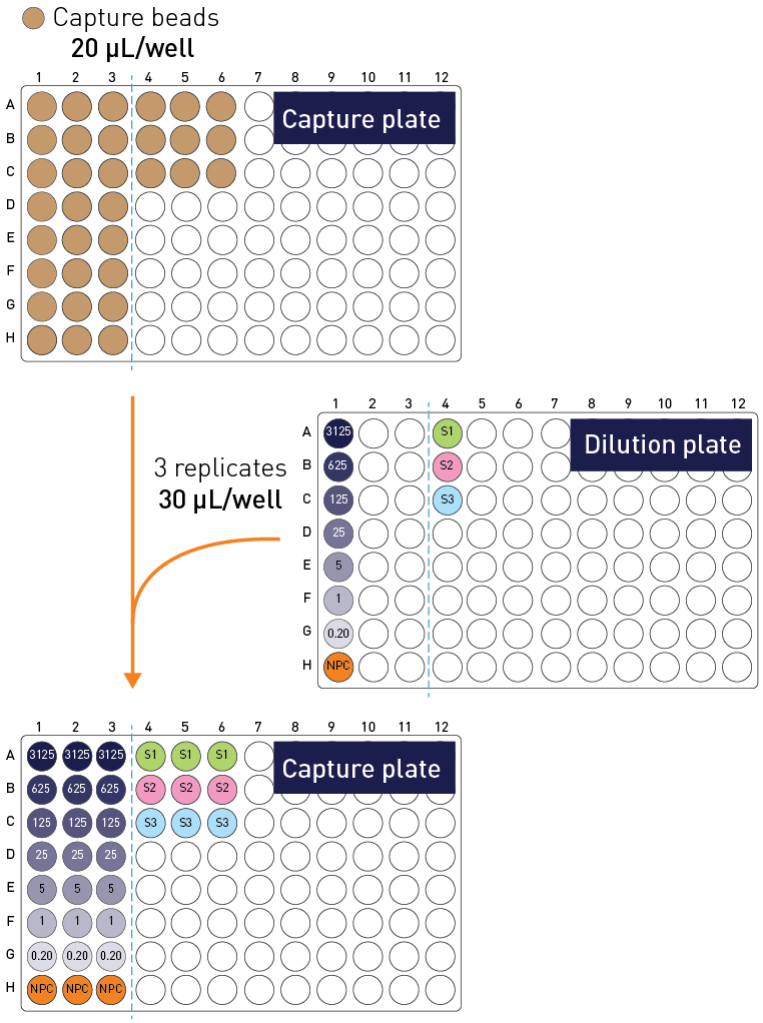


Figure 4 Transfer standards and samples to the capture plate

Run the plates in the magnetic particle processor

1. Turn on the instrument, then select the **CHO HCP** program from the screen.

   Note: The instrument automatically resets each time that you turn it on.

2. Press **START** to initiate plate loading. Follow the prompts on the display screen to load each plate onto the turntable, starting with "Comb" (see Figure 5). Slide each plate into the plate hold-down (if present).

IMPORTANT! When loading the **Tip Comb** in position 8, confirm that it rests in a MagMAX™ Express 96-well Standard Plate (200 µL; Cat. No. A31541), *not* a PCR Plate, 96-well, low-profile, skirted (Cat. No. AB-0800).

For all plates, verify that A1 on the plate aligns with A1 on the instrument.

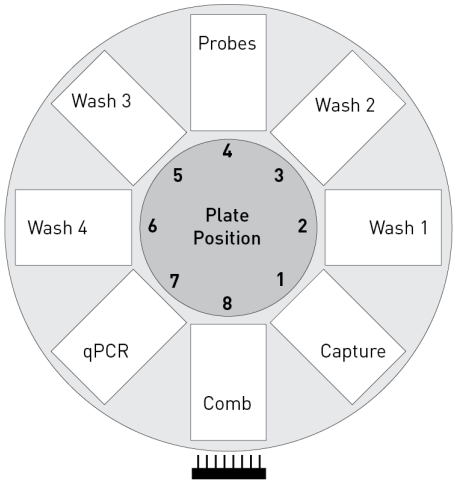


Figure 5 Plate positions in the turntable

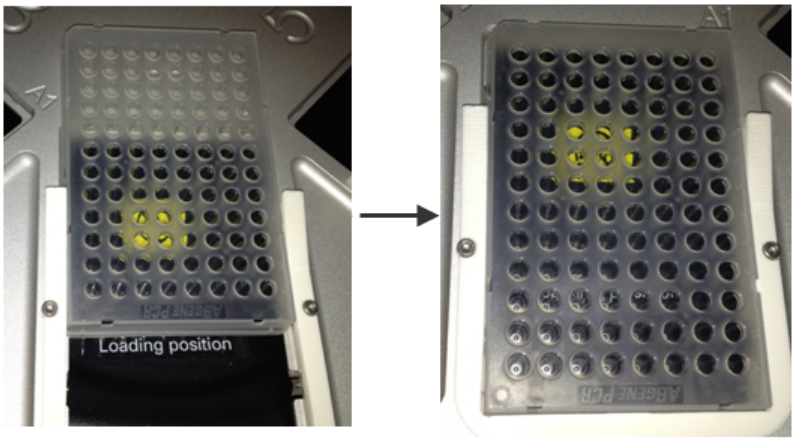


Figure 6 Load plate (with plate hold-down)

3. Load the last (Capture) plate, then press **START** to start the run.

   The run requires ~2 hours. When the run is complete, the screen displays "Proceed to qPCR".

Plate	Step
Capture	CHO HCP binds to Capture Beads
Wash 1	Capture Beads are washed
Wash 2	Capture Beads are washed
Probes	Probe binds to CHO HCP on Capture Beads
Wash 3	Capture Beads are washed
Wash 4	Capture Beads are washed
qPCR	Beads are released into qPCR plate

4. When the program is complete, carefully remove the qPCR plate. Discard the Capture, Wash, and Probes plates.

   IMPORTANT! Do not discard the plate adaptor.

   Note: Discard the PCR Head Tip Comb.

   The qPCR plate contains CHO HCP Capture Beads in ProteinSEQ™ Elution Buffer (total volume 15 µL (FAST plate) or 25 µL (standard plate).

Proceed immediately to "Run the qPCR reaction" on page 17.

Run the qPCR reaction

IMPORTANT! ProteinSEQ™ detection is based on qPCR, which is a highly sensitive technique with potential for cross-contamination. After the run completes, discard the qPCR plate. Do not remove the optical film from the qPCR plate. Removing the film introduces amplicon contamination into the local environment. See Appendix C, "Good laboratory practices for PCR and RT-PCR".

1. Prepare the Ligation/qPCR mix in a 15-mL tube according to the volumes shown in the appropriate table, including recommended overages, then briefly vortex to mix.

Table 4 Reagent volumes for FAST PCR plates.

Reagent	Cap color	Volumes[1]		
		1 rxn	48 rxn	96 rxn
Fast Master Mix, 2X	Clear	20 µL	960 µL	1920 µL
ProteinSEQ™ Ligation and Assay Mix[2]	Green	2 µL	96 µL	192 µL
ProteinSEQ™ Ligase	Orange	0.2 µL	9.6 µL	19.2 µL
Total		22.2 µL	1065.6 µL	2131.2 µL

[1] Includes 35% overage.
[2] Contains FAM™ dye and primers.

Table 5 Reagent volumes for standard (non-FAST) PCR plates.

Reagent	Cap color	Volumes[1]		
		1 rxn	48 rxn	96 rxn
Fast Master Mix, 2X	Clear	32.5 µL	1560 µL	3120 µL
ProteinSEQ™ Ligation and Assay Mix[2]	Green	3.25 µL	156 µL	312 µL
ProteinSEQ™ Ligase	Orange	0.26 µL	12.5 µL	25 µL
Total		36.01 µL	1728.5 µL	3457 µL

[1] Includes 30% overage. Volumes for 48 and 96 reactions are rounded to nearest tenth.
[2] Contains FAM™ dye and primers.

2. Transfer the Ligation/qPCR mix to each bead-containing well of the qPCR plate that was prepared on the instrument.

   - **For Fast PCR plates**—Use 15 µL per well
   - **For Standard PCR plates**—Use 25 µL per well

   Note: Dispense the mix to the sides of the well. Do not mix after dispensing.

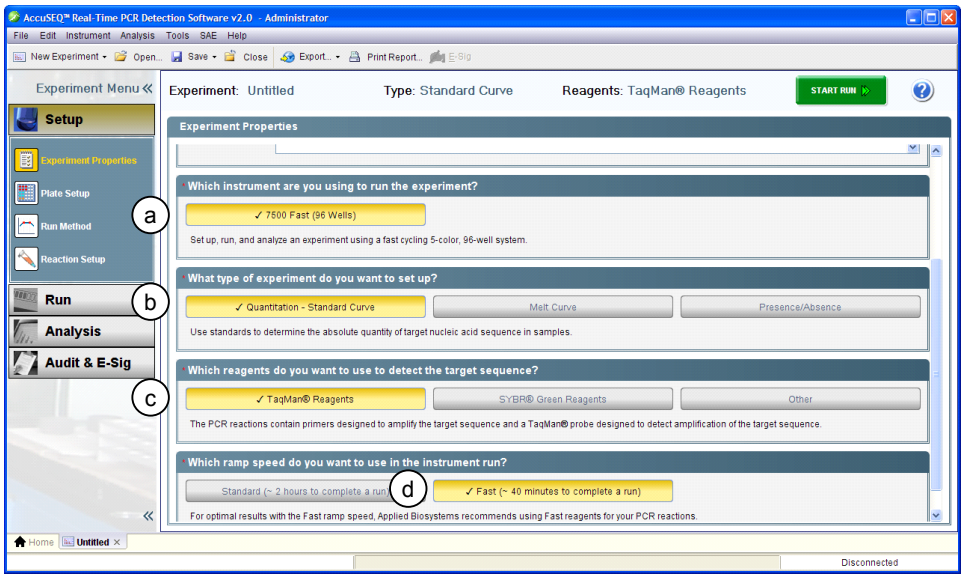
3. Seal the qPCR plate with an optical film, centrifuge for 3 seconds at 500 rpm, then load the plate on a 7500 Fast Real-Time PCR System (or equivalent).

4. Set up the qPCR run.
   - If you are using the AccuSEQ™ system software, follow the setup instruction in "Set up and run qPCR on the 7500 Fast instrument with AccuSEQ™ software" on page 18.
   - If you are using other equivalent software (for example, SDS 1.4 software) use the following settings:
Stage	Temperature	Time
Hold	37°C	10 minutes
Hold	95°C	20 seconds
40 cycles	95°C	3 seconds
	60°C	30 seconds
Setting	FAST plates	Standard (non-FAST) plates
CHO HCP standards and sample wells volume	30 µL	50 µL
Detection dye set	FAM	FAM
Quencher	none	none
5. Start the run.
6. After the run completes, discard the qPCR plate. Do not remove the optical film from the qPCR plate. Removing the film introduces amplicon contamination into the local environment.

Set up and run qPCR on the 7500 Fast instrument with AccuSEQ™ software

1. From the home screen click **Create Custom Experiment**.
2. Make the following selections in the Experiment Properties Pane:
   a. 7500 Fast (96 Wells)
   b. Quantitation–Standard Curve
   c. TaqMan® Reagents

d. Fast (~ 40 minutes to complete a run)



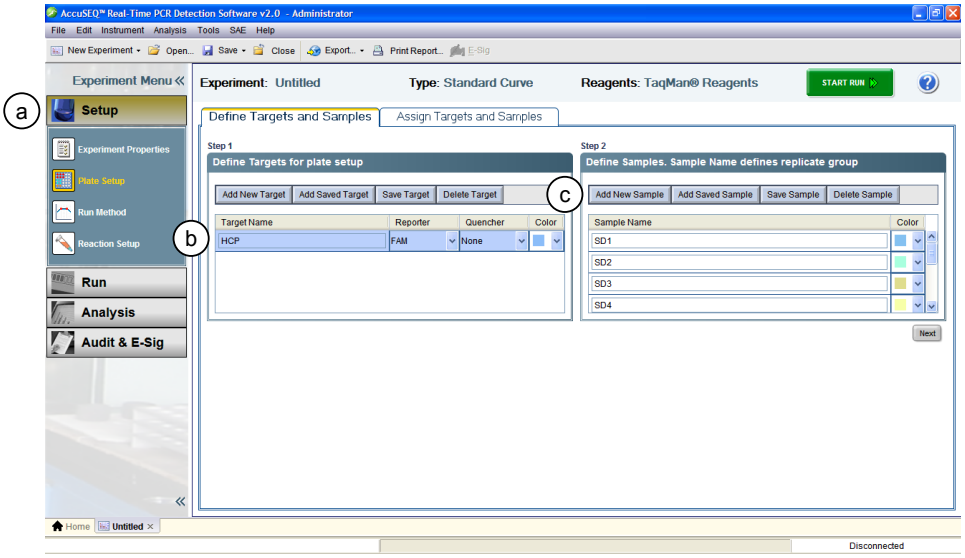
3. Define Sample Number and Name.

   a. Click **Plate Setup** in the Experiment Menu Pane.

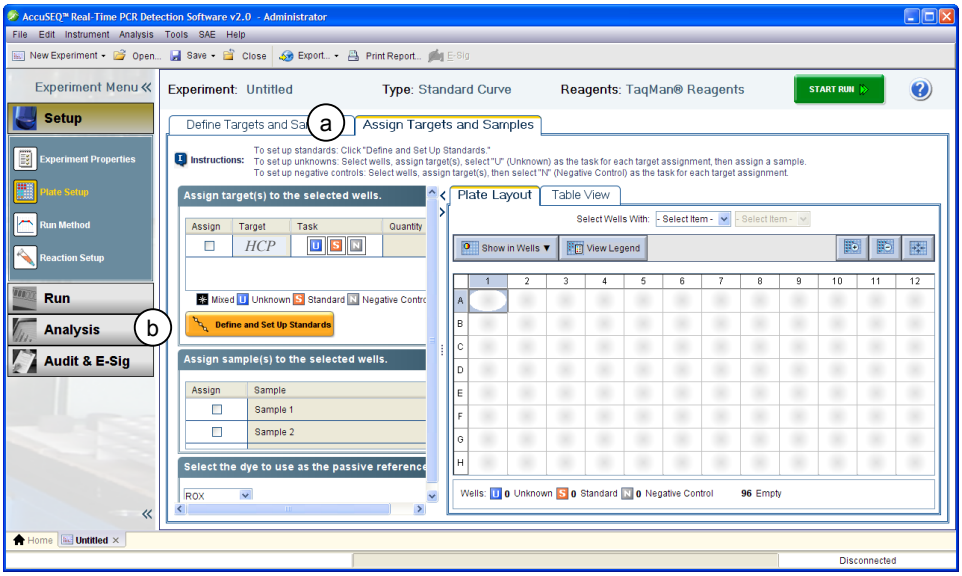
   b. Enter **CHO HCP** as the target name, select **FAM** as the reporter and **None** as the quencher.

   c. Enter the number and name of your samples, excluding replicates. Click **Add New Sample** to enter the number of samples to be run.

   For example, if you have four samples run in triplicate, you would define four samples in this step. Replicates of those four samples will be defined in the next step.

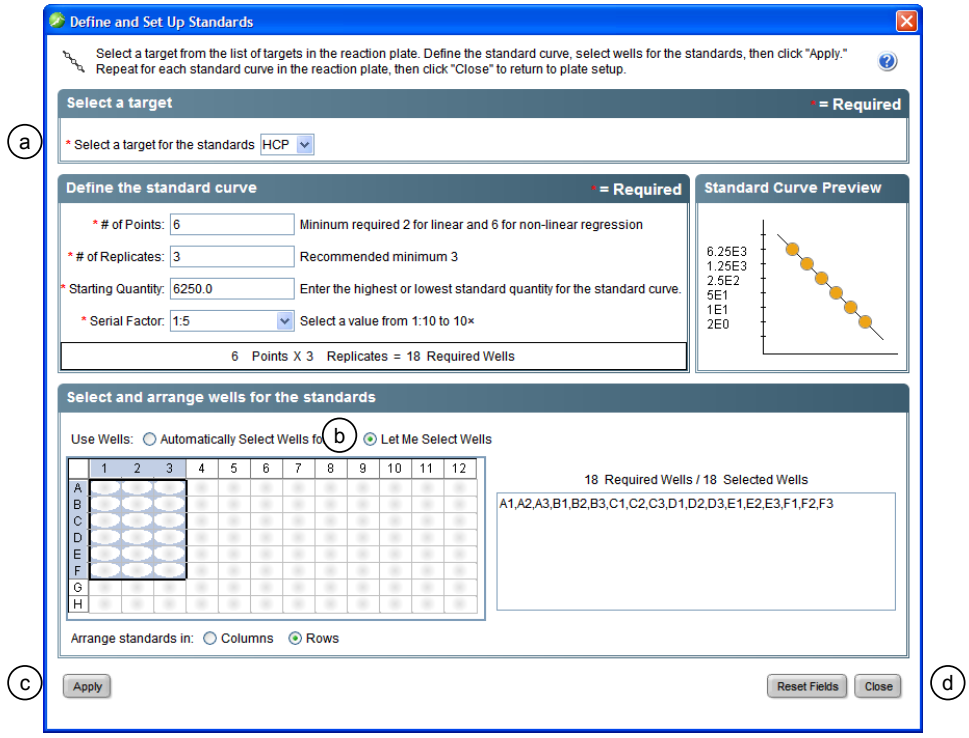


4. Access the Standard Curve Dialog as follows:
   a. Select the **Assign Targets and Samples** tab.
   b. Click **Define and Setup Standards**.



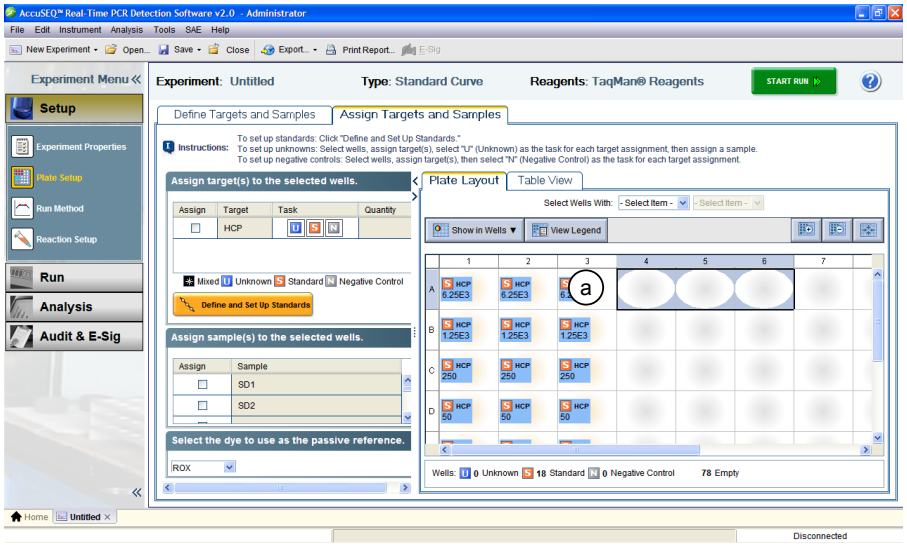
5. Setup the Standard Curve as follows:
   a. In the Define the standard curve tab, enter **7** for "# of Points", **3** for "# of Replicates", **3,125** for "Starting Quantity", and **1:5** for "Serial Factor".
   b. Click **Let Me Select Wells**. Click, hold, and drag the plate map to select the wells to be used as standards.
   c. Click **Apply**.

d. Click **Close**.

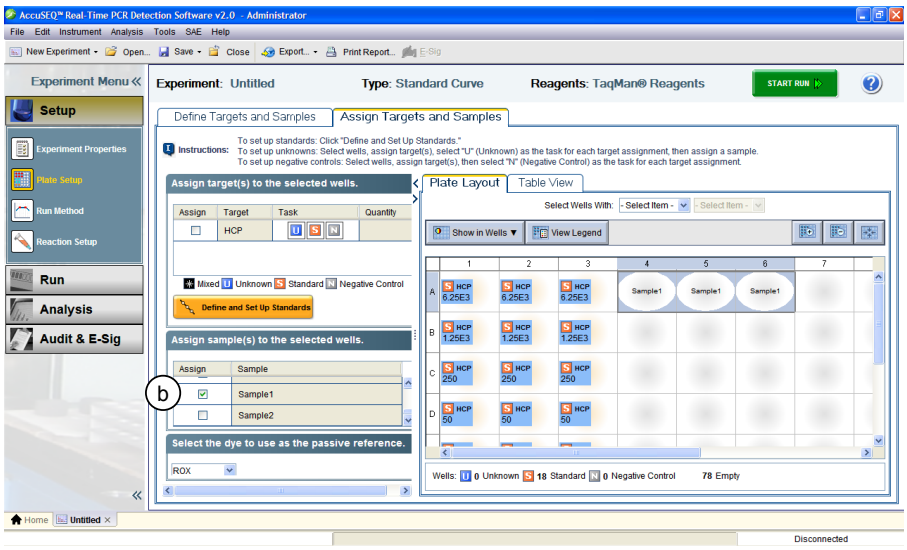


6. Assign sample name to wells.

   a. In Plate Layout, select all wells that will be assigned as replicates for Sample 1. In this example, A4, A5 and A6 are selected.

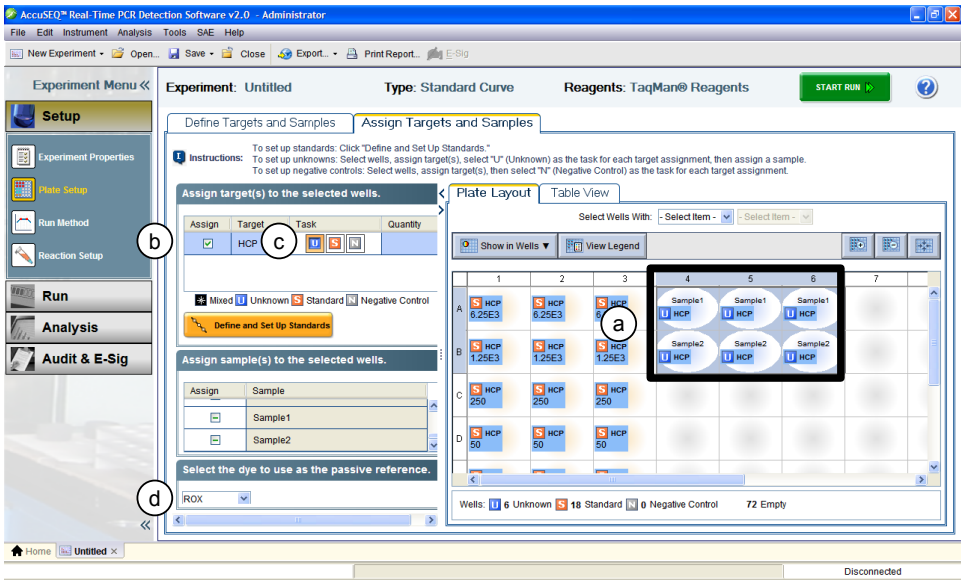


b. Click **Assign** next to the appropriate sample. Repeat for all unknown samples.



In this example, “Sample 1” is assigned to wells A4, A5 and A6.

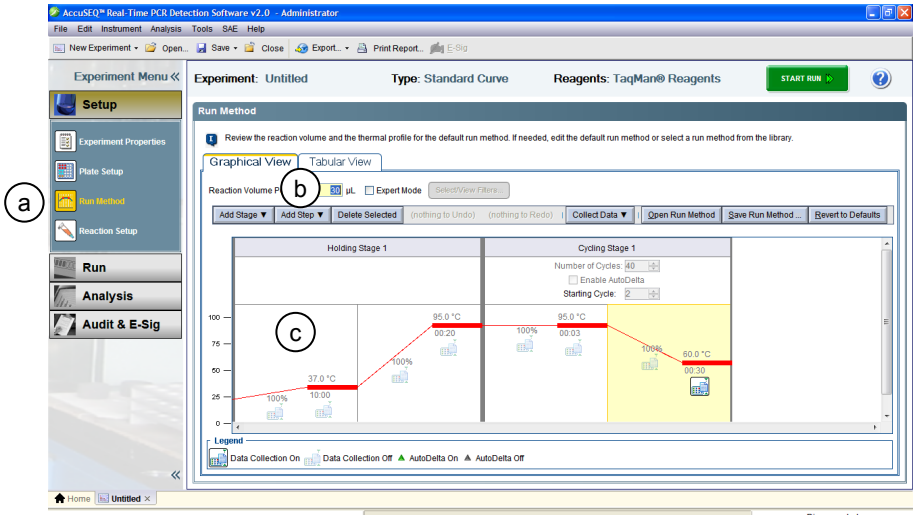
7. Assign Unknown Well Type as follows:
   a. Select all wells that will be designated as unknowns.
   b. Click **Assign** under Assign Targets to the Selected Wells.
   c. Click the blue **U** to assign the wells as unknowns.
   d. Verify that **ROX** is selected as the passive reference.



8. Setup qPCR parameters as follows:
   a. Click **Run Method**.

b. Enter a reaction volume of **30 µL**.

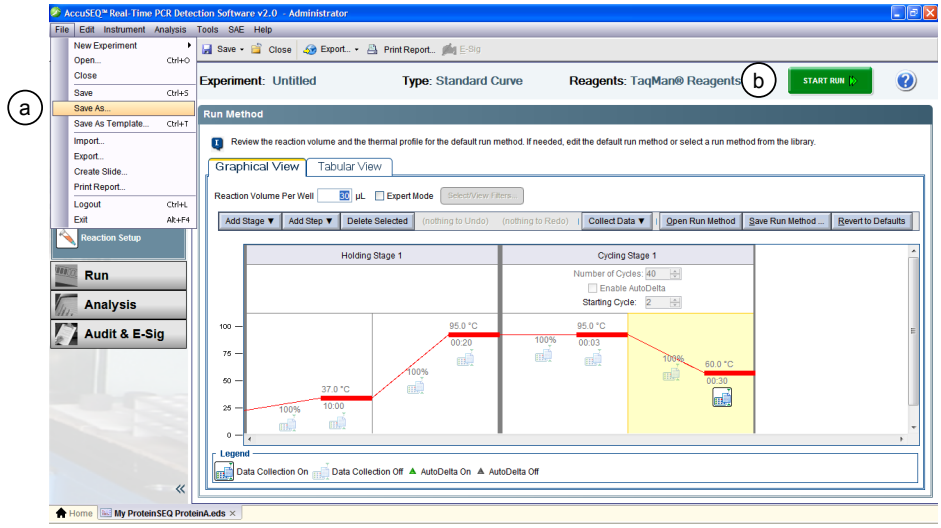
c. Verify reaction parameters match those shown:



9. Save the setup as follows:

a. Select **File ▸ Save As...** (or select **File ▸ Save As Template...** to save this experimental setup for future use).

b. Click **Start Run**.



Perform data analysis

Perform data analysis with AccuSEQ™ software v2.0 or later

1. In the AccuSEQ™ software, select autobaseline **on**, then set the threshold manually to 0.2.
2. Use the AccuSEQ™ software to fit standards to a curve using a nonlinear method, then obtain interpolated values for the unknowns.
   - 4PL is commonly used for symmetric curves with asymptotes for both the lower and upper CHO HCP concentrations.
   - 5PL is commonly used if the curve is asymmetric or if either the lower or upper asymptote is not present.
   - Apply 1/Y or 1/Y^2 weighting according to your criteria.
3. Export the data to a Microsoft™ Excel™ spreadsheet for custom statistical analysis.
4. Evaluate the dynamic range using %CV and the quality of the curve fit.
   - R^2 is appropriate for judging linear fits but it is not an appropriate metric for evaluating the quality of a non-linear fit.
   - Common acceptance criteria for non-linear curve fits are back-calculation values of 80–120% throughout the curve and 75–125% at the LLOQ.
   - Common acceptance criteria for precision are %CV ≤20% throughout the curve and≤25% at the LLOQ.
5. Obtain the final concentration for each sample by correcting for sample dilution and spike concentrations, if used.

Perform data analysis without AccuSEQ™ software

1. Select autobaseline **on**, then set the C_t threshold manually to **0.2**. Determine the C_t values.
2. Export the raw data from the qPCR software to a Microsoft™ Excel™ spreadsheet, then export from Microsoft™ Excel™ to your fitting program of choice. Transform the values to logarithmic values.

   Note: If you use GraphPad™, the CHO HCP Master Template (a Microsoft™ Excel™ template available from your local sales or service representative) helps this process.
3. Fit standards to a curve using a non-linear method, then obtain interpolated values for the unknowns.
   - 4PL is commonly used for symmetric curves with asymptotes for both the lower and upper CHO HCP concentrations.
   - 5PL is commonly used if the curve is asymmetric or if either the lower or upper asymptote is not present.
   - Apply 1/Y or 1/Y^2 weighting according to your criteria.
4. Transform concentration values from logarithmic to linear values.

5. Evaluate the dynamic range using %CV and the quality of the curve fit.
   - R^2 is appropriate for judging linear fits but it is not an appropriate metric for evaluating the quality of a non-linear fit.
   - Common acceptance criteria for non-linear curve fits are back-calculation values of 80–120% throughout the curve and 75–125% at the LLOQ.
   - Common acceptance criteria for precision are %CV ≤20% throughout the curve and ≤25% at the LLOQ.
6. Obtain the final concentration for each sample by correcting for sample dilution and spike concentrations, if used.

A Troubleshooting

Observation	Possible cause	Recommended action
Capture beads remain on comb	Misalignment of the magnetic head.	Contact your local Technical Support for re-alignment of the instrument.
	qPCR plate not placed in appropriate PCR Plate Adaptor during the magnetic particle processor instrument run.	Use the appropriate PCR Plate Adaptor (Fast or Standard; request from your local sales or service representative).
The standard curve plateaus at the lower standard concentrations and the NPC C_T is less than 28	Cross-contamination of CHO HCP or ligated product.	Decontaminate the bench and pipettors. Change gloves frequently and follow other good PCR practices. After the run completes, dispose of the qPCR plate. Do not remove the optical film from the qPCR plate; removing the film introduces amplicon contamination into the local environment. See Appendix C, "Good laboratory practices for PCR and RT-PCR". Before preparing the plates for each assay, clean the pipettors, plate racks and the microcentrifuge (if using tubes for standard dilution) to avoid cross-contamination.
		If prone to contamination, change the order of standards, sample, and plate preparation as follows: • Label plates • Prepare wash plates • Prepare probes plate • Move the prepared plates near the magnetic particle processor. • Prepare standards and samples. • Prepare capture plate
	The reagents are contaminated.	Use new reagents.
The C_T at 3,125 pg/mL is above 20 and the NPC C_T is undetermined	Expired kit.	Check kit expiration date.
	Errors in reaction or run setup.	Repeat assay preparation. Make sure that the components are added in the recommended order.
Trending increase in C_T value for standard concentrations from run to run	Deterioration of standards.	Prepare fresh standards. Verify kit expiration date.
Random decrease in C_T during run	Cross-contamination of concentrated standards or samples with lower concentration samples.	Repeat experiment.

Observation	Possible cause	Recommended action
Random failures across the plate	Air bubbles introduced into plate wells during plate setup.	Dispense at bottom of wells to prevent bubble formation (bubbles prevent effective mixing during the magnetic particle processor run). If bubbles form, quick-spin the plate at 560 × g (~2000 rpm) in a plate centrifuge.
Poor recovery and/or efficiency during spike experiments	• Incorrectly designed spike amount. *or* • Sample concentration is higher than expected.	Use a spike amount 50–100% of the concentration in the unspiked sample. See Appendix B, "Design guidelines for ProteinSEQ™ System HCP spike experiments".
Low spike efficiency	Salt concentration in sample well is too high.	Pre-dilute the sample so that final concentration of salt in the reaction well is <50 mM.
	Matrix interference from IgG or other components.	Evaluate the assay performance with higher sample dilutions.
Increased percent CV	Incorrect plate type used.	Use PCR Plates, 96-well, low profile, skirted, (Cat. No. AB-0800) for Capture, Probes, and Wash plates. See "Label the plates" on page 12.

B Design guidelines for ProteinSEQ™ System HCP spike experiments

About spike experiments

Spike recovery and efficiency experiments are essential tools for evaluating the accuracy of a quantitation assay in relevant matrices.

Note: A spike experiment that includes sample preparation steps is referred to as a "recovery" experiment. An experiment without sample preparation steps is referred to as an "efficiency" experiment.

A basic spike recovery experiment includes these steps. See Figure 7 on page 29.

1. If necessary, dilute the sample according to the experimental goal.
2. Split the starting sample matrix into two aliquots, one for spiking and a second for referencing.
3. Add a known amount of analyte (e.g. stock from the standard curve dilution series) into the spike sample, and add a volume of sample diluent (e.g. buffer) equal to the spike volume to the reference sample.
4. Analyze the spike and the reference sample using the same method to generate a mean observed quantitation value.
5. In data processing, subtract the Mean Quantity (Reference) from the Mean Quantity (Spike) to calculate the Reference Adjusted Quantity (RAQ).
6. Divide the RAQ by the Spike Input and multiply by 100 to arrive at a Percent Recovery or Efficiency (HCP experiments are reported as "Percent Efficiency").

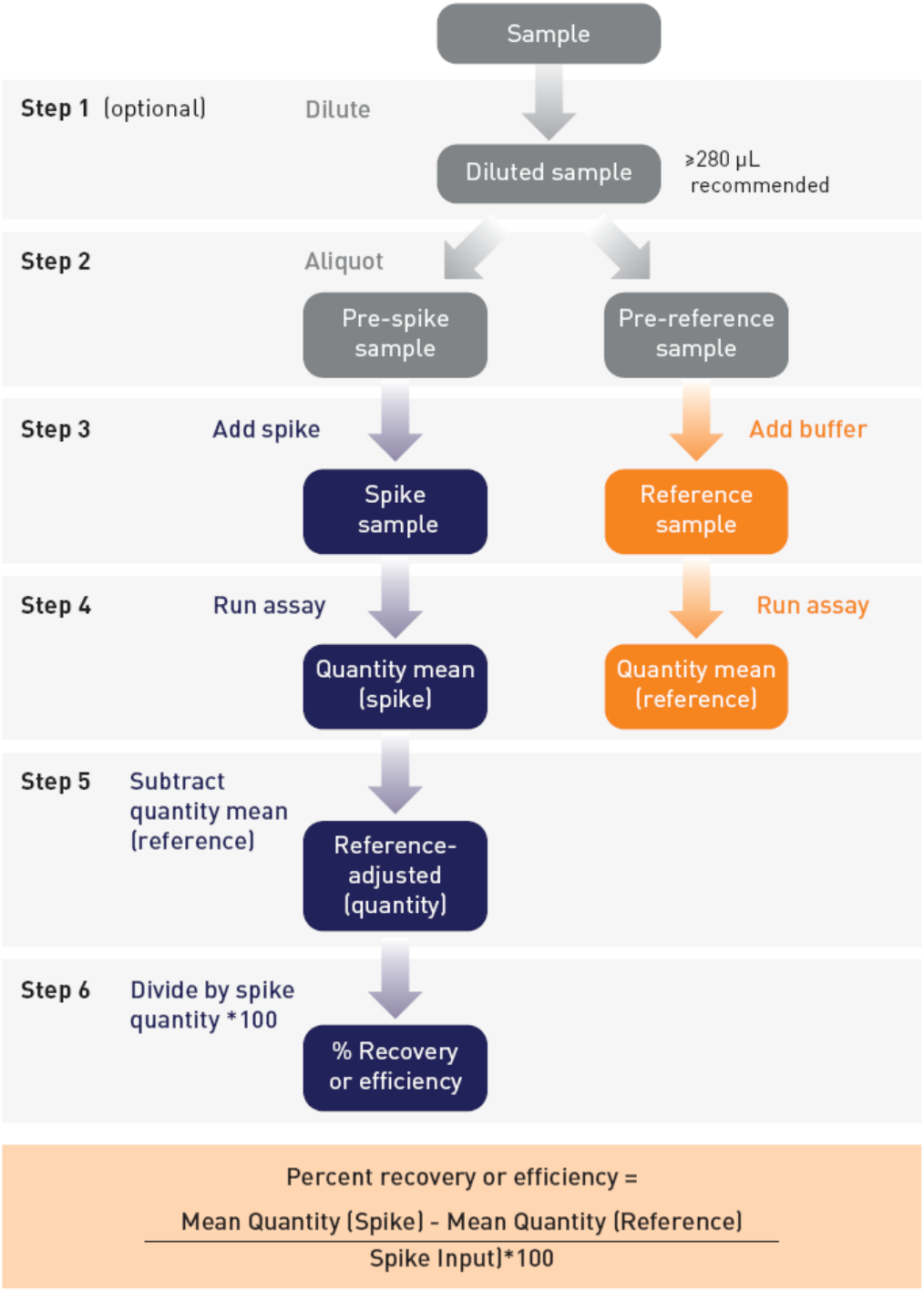


Figure 7 Use of a spike and reference sample to determine percent efficiency for a quantitation assay

Important experimental design considerations

To obtain informative and valid results, consider the following when designing the experiment:

- What concentration of analyte in the spike should be evaluated?
- At what ppm (drug product concentration) should the analyte be evaluated?
- What matrices are available for evaluation?

Guidelines for spike input concentration

The spike input concentration is defined by the goals of the experiment, the concentration of the analyte in the reference sample and the position of that concentration within the standard curve.

The concentration of the spike must be large enough to be differentiated from the analyte concentration already present in the reference sample. Typical spike concentrations range from 50–100% of the reference concentration. Therefore, the reference sample analyte concentrations must be known in order to select the proper spike input concentration.

- If the reference analyte concentration lies between the LLOQ and the mid-point of the standard curve, a 100% spike is recommended.
- If the reference analyte concentration is above the midpoint of the standard curve, a 50% spike is recommended. Note that working at the upper end of standard curve requires care that the final concentration after spiking does not exceed the ULOQ.

Guidelines for matrix selection

The choice of matrix directly affects the design of a spike experiment due to the fact that the ratio of analyte to drug (expressed as ppm in ng analyte/mg drug substance) is a fixed ratio that does not change with dilution. Therefore, practical limitations exist for working with all matrices and a priority must typically be assigned to evaluate either a specific analyte concentration (and the drug substance concentration that follows) or a specific drug substance concentration (and the analyte concentration that follows). For this reason, it is recommended that the goal of the experiment be established followed by procurement of a matrix rather than vice versa.

As an example of the impact of the matrix on design of an HCP experiment, consider a matrix containing 10,000 ng/mL HCP and 10 mg/mL of drug substance (1,000 ppm). If priority is assigned to measuring HCP at 10 ng/mL, then experimental design would require a 1,000X dilution — consequently reducing the IgG concentration to 0.01 mg/mL. Such an experiment would evaluate HCP quantitation accuracy at 10 ng/mL but it would reveal little about the interference of matrix components (drug substance, salts, detergents) because the extensive dilution would reduce their concentrations to very low levels. For this reason, when both accuracy and interference are to be evaluated, late process samples containing lower analyte concentrations are typically more conducive to spiking studies than early process samples. For example, a late process sample matrix containing 100 ng/mL HCP and 10 mg/mL IgG (10 ppm) would require only a 10X dilution to investigate efficiency at

10 ng/mL, leaving the drug substance at a reasonable concentration of 1 mg/mL and therefore would provide information on both accuracy and interference.

Volume of sample		115	115	115
Volume of spike		5	10	15
Stock concentrations for spiking	15,625	651.0	1250.0	1802.9
	3,125	130.2	250.0	360.6
	625	26.0	50.0	72.1
	125	5.2	10.0	14.4
	25	1.0	2.0	2.9
	5	0.2	0.4	0.6
	1	0.0	0.1	0.1
	0.2	0.0	0.0	0.0

Figure 8 Final HCP concentration of spike using various standard curve stock concentrations and volumes. Green cells = concentrations recommended for spiking studies. Yellow cells = concentrations within the dynamic range but not recommended for spiking studies. Red cells = concentrations out of the ProteinSEQ™ standard curve dynamic range.

Example experiments

HCP quantitation example 1

Experimental goals: Evaluate HCP quantitation at ~20 ng/ml in the presence of ~1 mg/mL IgG. An evaluation matrix containing an estimated 100 ng/mL of HCP and 10 mg/mL IgG is procured.

1. Dilute 10X by mixing 30 µL sample with 270 µL Sample Diluent to reach a recommended volume of >280 µL. The expected concentrations become 10 ng/mL HCP and 1 mg/mL IgG. Note that the concentration of the analyte in the matrix after dilution is below the target concentration to be evaluated.
2. Generate a Pre-Reference aliquot of 115 µL and a Pre-spike aliquot of 115 µL in the Dilution Plate. The remaining volume may be discarded.
3. Spike the sample. Figure 8 on page 31 indicates that a 100% spike of 10 ng/mL may be achieved by spiking 115 µL of sample with 10 µL of the 125 ng/ stock solution. To create a matching reference, 115 µL of matrix is also combined with 10 µL of Sample Diluent to prepare the Reference Sample. Replicate 30 µL of the spiked sample in triplicate in the Capture plate. The remaining volume can be discarded.
4. Run the assay to obtain values for Mean Quantity (Spiked) and Mean Quantity (Reference). In this example, the observed values for Mean Quantity (Spiked) and Mean Quantity (Reference) are 19.0 ng/mL and 8.0 ng/mL respectively.

5. Calculate the Reference Adjusted quantity for the spiked sample by subtracting Mean Quantity (Reference) from Mean Quantity (Spiked).
6. Calculate Percent Recovery by dividing the Reference Adjusted quantity by the Spike Input and multiplying by 100.

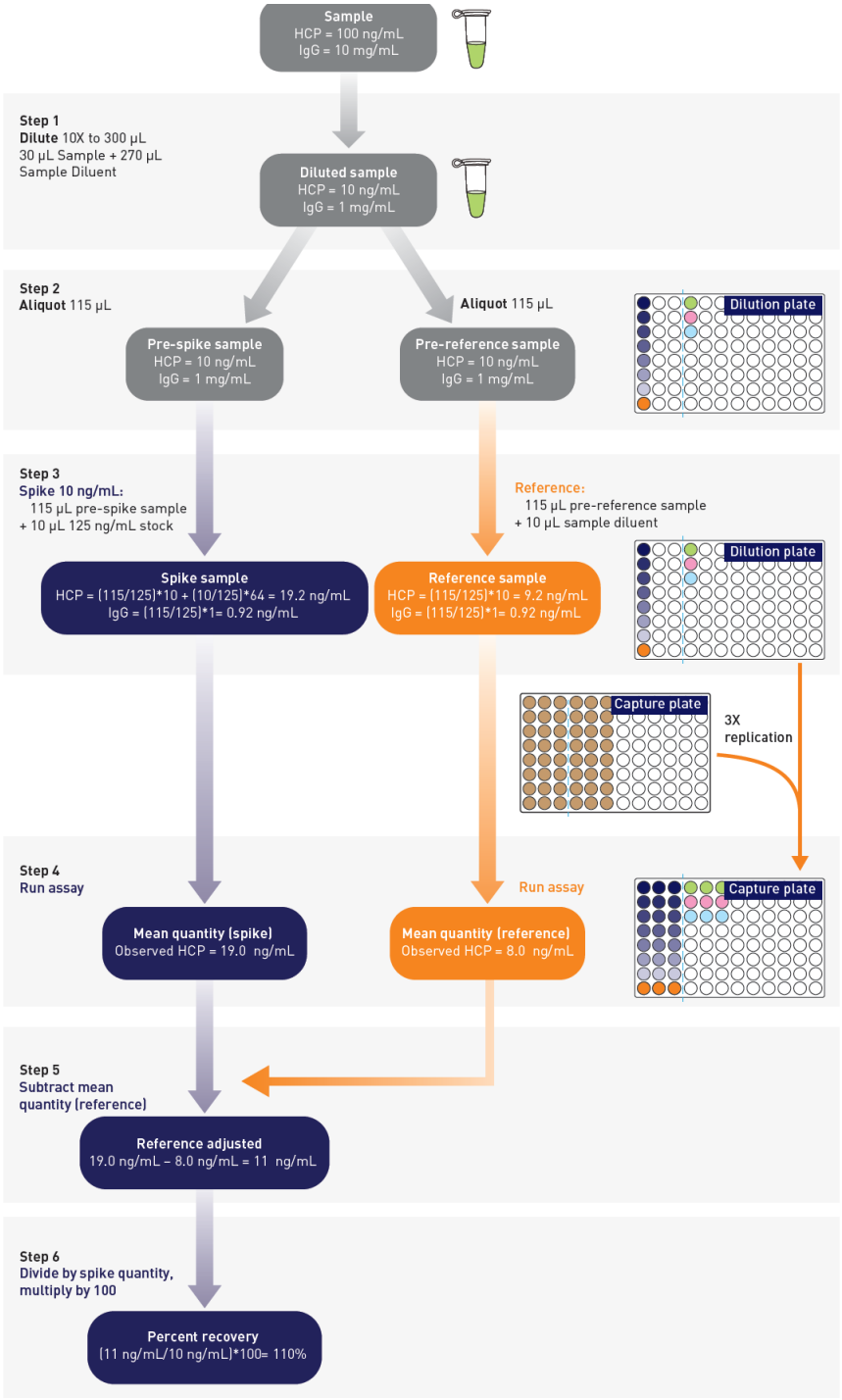


Figure 9 Example 1 for HCP spike experiment with calculations.

HCP quantitation example 2

Experimental goals: Evaluate HCP quantitation at ~100 ng/ml in the presence of ~2 mg/mL IgG. An evaluation matrix containing an estimated 500 ng/mL of HCP and 4 mg/mL IgG is procured. Note that in this example the goal is to evaluate the assay at 100 ng/mL HCP and 2 mg/mL IgG, which is 50 ppm but the available matrix is 100 ppm. Since the ppm of the matrix is greater than that of the experimental goal, some compromise must be made in the experimental design as priority must be placed on evaluating either the HCP at 100 ng/ mL or IgG at 2 mg/mL—it's not possible to do both. In this example, the decision is made to prioritize interference at an IgG concentration of 2 mg/mL.

1. Dilute 2X by mixing 150 µL sample with 150 µL Sample Diluent to reach a recommended total volume of ≥280 µL. The expected concentrations become 250 ng/mL HCP and 2 mg/mL IgG.
2. Generate a Pre-Reference aliquot of 115 µL and a Pre-spike aliquot of 115 µL in the Dilution Plate. The remaining volume may be discarded.
3. Spike the sample. Figure 8 on page 31 indicates that a 100% spike of 250 ng/mL may be achieved by spiking 115 µL of sample with 10 µL of the 3125 ng/mL stock solution. In order to have a matching reference, 115 µL of matrix is also combined with 10 µL of Sample Diluent to prepare the Reference Sample. Replicate 30 µL of the spiked sample in triplicate in the Capture plate. The remaining volume can be discarded.
4. Run the assay to obtain values for Mean Quantity (Spiked) and Mean Quantity (Reference). In this example, the observed values for Mean Quantity (Spiked) and Mean Quantity (Reference) are 490 ng/mL and 260 ng/mL respectively.
5. Calculate the Reference Adjusted quantity for the spiked sample by subtracting Mean Quantity (Reference) from Mean Quantity (Spiked).
6. Calculate Percent Recovery by dividing the Reference Adjusted quantity by the Spike Input and multiplying by 100.

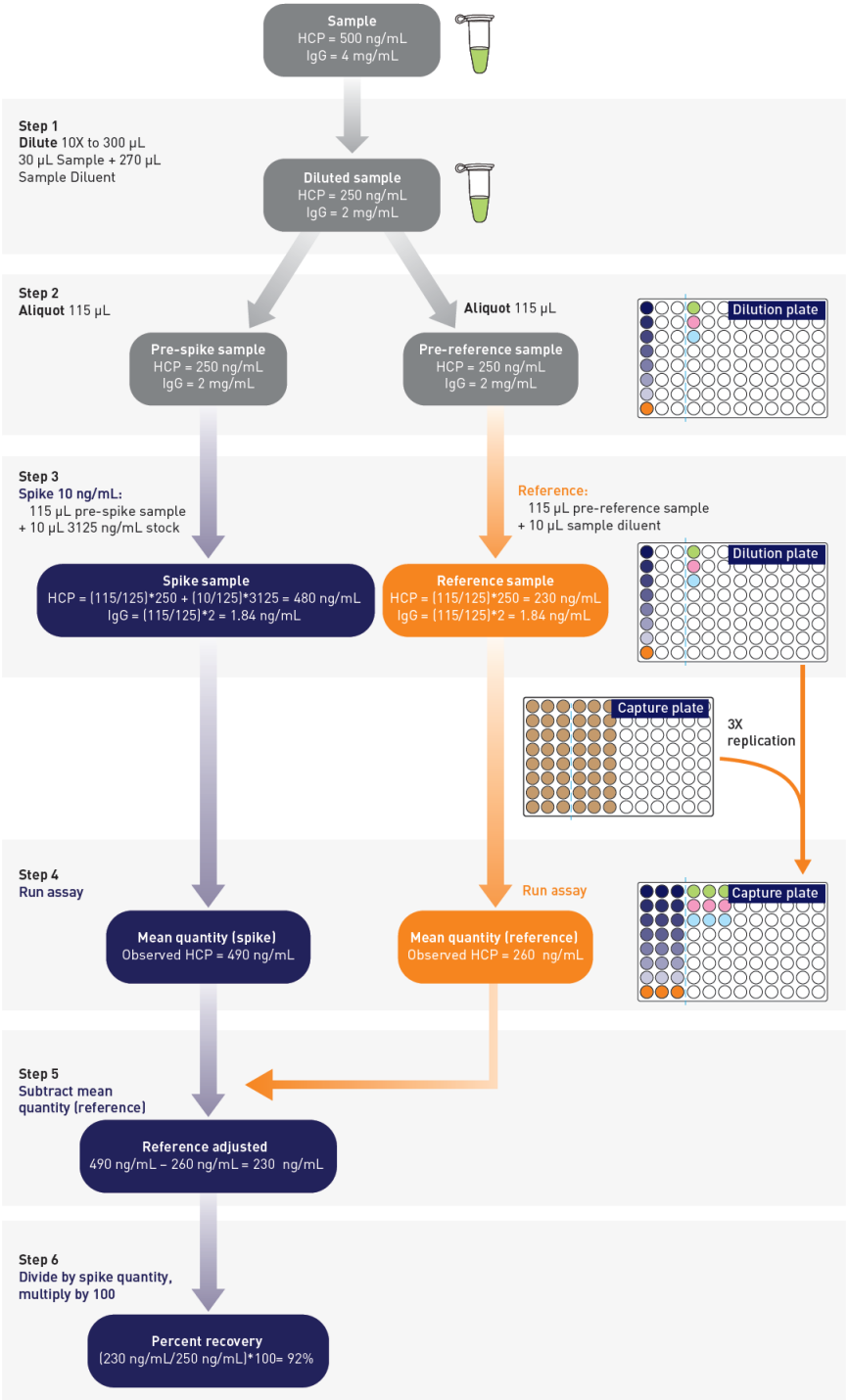


Figure 10 Example 2 for HCP spike experiment with calculations.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used 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

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Portable document format (PDF) versions of this guide and the following related documents are available from **thermofisher.com/support**:

Document	Publication number	Description
ProteinSEQ™ CHO HCP Quantification KIt Quick Reference — Workflow for FAST PCR plates	MAN0010252	Provides information on preparing and running assays using FAST PCR plates.
ProteinSEQ™ CHO HCP Quantification KIt Quick Reference — Workflow for Standard (non-FAST) PCR plates	MAN0010251	Provides information on preparing and running assays using Standard (non-FAST) PCR plates.

Note: To open the user documentation, use the Adobe™ Reader™ software available from **www.adobe.com**

Note: For additional documentation, see "Customer and technical support" on page 39.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.