ProteinSEQ™ CHO HCP Quantification Kit

Workflow for Standard (non-FAST) PCR plates

Catalog Number A27601

Pub. No. MAN0010251 **Rev.** B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ProteinSEQ™ CHO Host Cell Protein Quantification Kit User Guide* (Pub. no. MAN0010806). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Procedures

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 the *ProteinSEQ™ CHO Host Cell Protein Quantification Kit User Guide* 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.

Prepare serial dilutions of the CHO HCP standard

1. Dispense 160 µL CHO HCP ProteinSEQ™ Diluent to each of the eight tubes or plate wells (see Figure 1 on page 1).
2. Serially dilute the CHO HCP standard from SD1 to SD7 (tubes) or A1 to G1 (plate wells, which are shown in Figure 2).

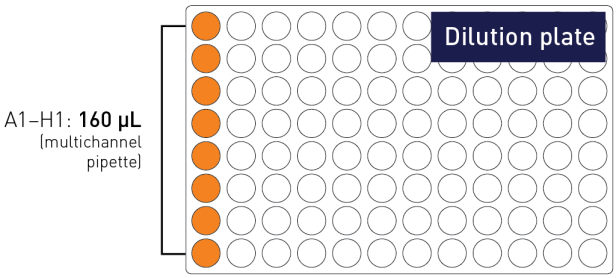


Fig. 1 Dispense CHO HCP ProteinSEQ™ diluent (shown in plate format)

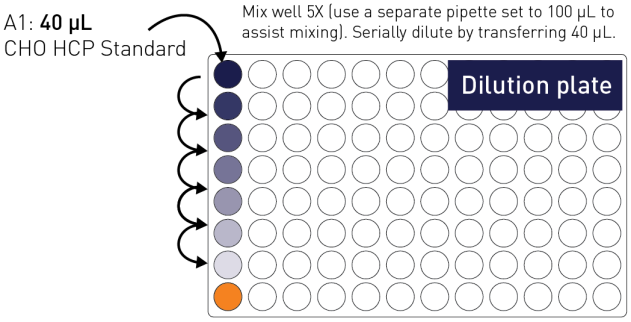


Fig. 2 Serially dilute the CHO HCP standard from SD1 to SD7 (tubes) or A1 to G1 (shown in plate format)

Prepare diluted samples

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

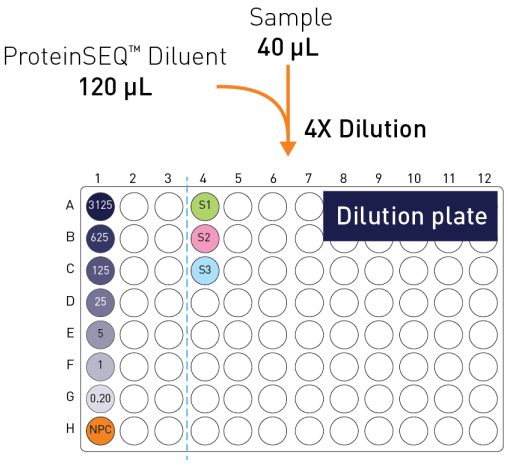
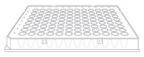
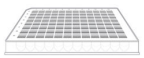


Fig. 3 Dilute samples (example 4X dilution, shown in plate)

Prepare the plates for the run

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™ Optical 96-Well Reaction Plate with Barcode (0.2 mL)		4306737	qPCR
1	MagMAX™ Express-96 Standard Plate (200 µL)		A31541	Comb

2. Insert the plate labeled "qPCR" into a Standard PCR Plate Adapter (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

Dispense 100 µL of Wash Buffer into each well of the 4 wash plates with a multi-channel pipette.

Prepare qPCR plate

Dispense 25 µL of ProteinSEQ™ Elution Buffer into each well of the qPCR plate.

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. Vortex and 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, then immediately dispense 20 µL into each well of the Capture plate.
2. 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.

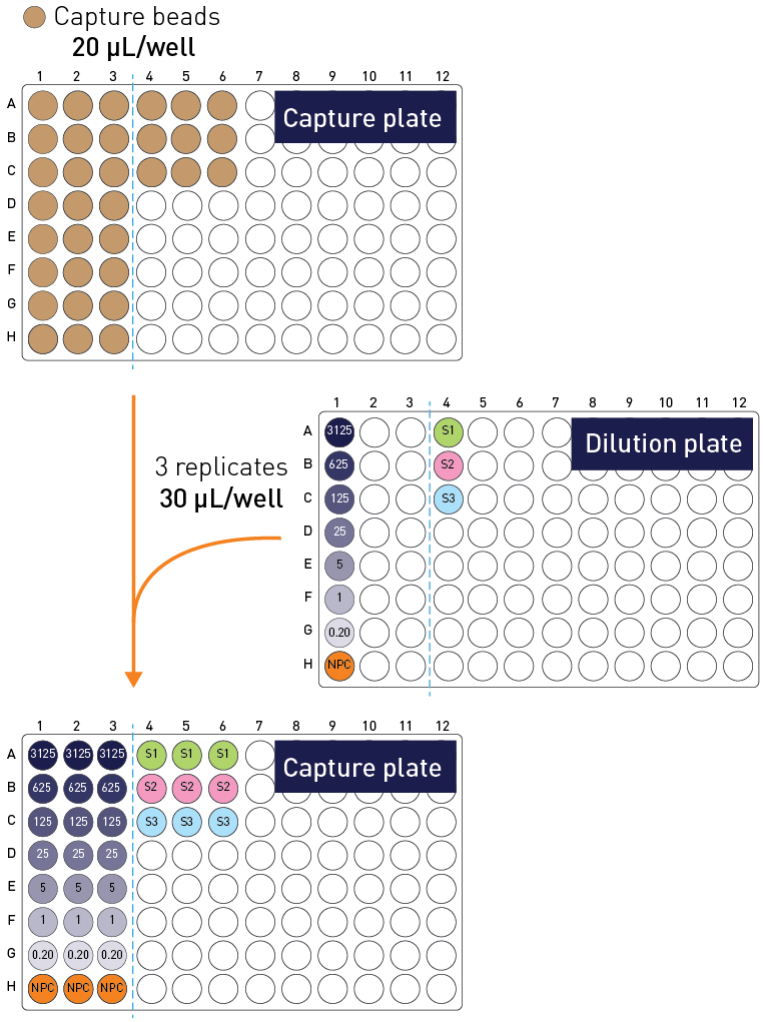


Fig. 4 Transfer standards and samples to the capture plate

Run the plates in the magnetic particle processor

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

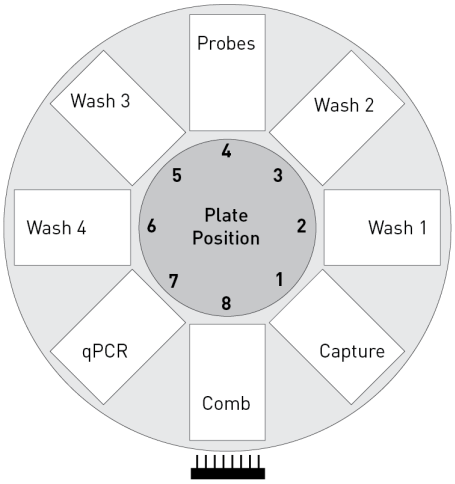


Fig. 5 Plate positions in the turntable

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

Proceed immediately to "Run the qPCR reaction" on page 2.

Run the qPCR reaction

1. Prepare the Ligation/qPCR mix in a 15-mL tube, then briefly vortex to mix.

Table 1 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	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. Transfer 25 µL of the Ligation/qPCR mix to each bead-containing well of the qPCR plate that was prepared on the MagMAX™ Express-96 instrument.
3. Seal the qPCR plate with an optical film, centrifuge for 3 seconds at 500 rpm, then load the plate on a 7500 Real-Time PCR System (or equivalent).
4. Set up the run using AccuSEQ™ system software (or equivalent, for example, SDS 1.4 software).

Stage	Temperature	Time
Hold	37°C	10 minutes
Hold	95°C	20 seconds
40 cycles	95°C	3 seconds
	60°C	30 seconds

- Set the CHO HCP standards and sample wells to a volume of **50 µL** with detection dye set to **FAM** and the quencher set to **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.

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.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision history: Pub. No. MAN0010251

Revision	Date	Description
B.0	4 February 2019	Update template and legal information.
A.0	8 December 2014	New document.