applied biosystems
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ProteinSEQ™ CHO Host Cell Protein Quantification Kit

Workflow for FAST PCR plates

Catalog Number A27601

Pub. No. MAN0010252 **Rev.** A.0

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

Contents	Cap color	Storage conditions
CHO HCP Standard (15.6 µg/mL)	Blue	–20°C Store at 4°C after thawing.[1]
CHO HCP 5′ Probe	Grey	–20°C
CHO HCP 3′ Probe	Yellow	
ProteinSEQ™ Ligation and Assay Mix	Green	
ProteinSEQ™ Ligase	Orange	
Fast Master Mix, 2X	Clear	4°C
Wash Buffer	Clear	
CHO HCP Capture Beads	Clear	
ProteinSEQ™ Elution Buffer	Clear	
CHO HCP ProteinSEQ™ Diluent	Clear	

[1] After thawing, do not re-freeze. Store at 4°C for up to 1 month.

Note: For safety and biohazard guidelines, refer to 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.

Important procedural guidelines

- The MagMAX™ Express-96 PCR Well Magnetic Head is fragile. Handle with care.
- It is critical to mix standards during serial dilution. After each transfer, invert the tube several times to mix, or, if preparing in a 96-well plate, gently pipet up and down 5-8 times to increase mixing efficiency.
- Use serially diluted standards when performing spiking studies. See *ProteinSEQ™ CHO Host Cell Protein Quantification Kit User Guide* (Pub. no. MAN0010806) for spiking guidelines.

Step 1: 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).
2. Serially dilute the CHO HCP standard from SD1 to SD7 (tubes) or A1 to G1 (plate wells, shown in Figure 2).

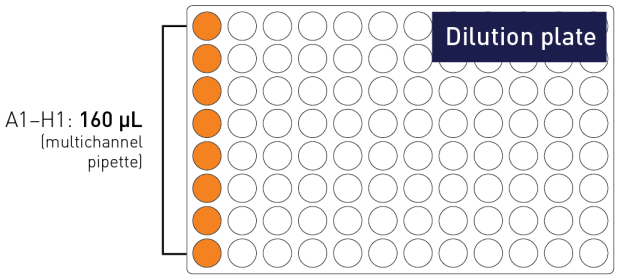

Figure 1 Dispense CHO HCP ProteinSEQ™ Diluent (shown in plate format)

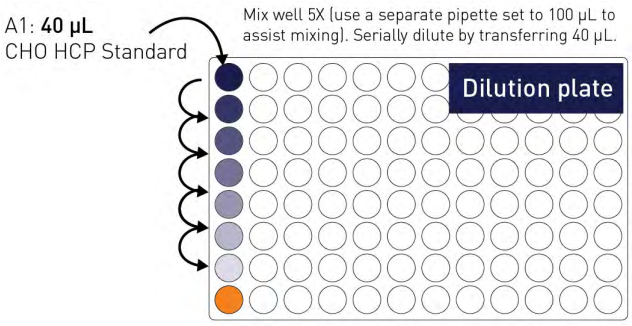

Figure 2 Serially dilute the CHO HCP standard from SD1 to SD7 (tubes) or A1 to G1 (plate format, shown)

Step 2: Prepare diluted samples

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

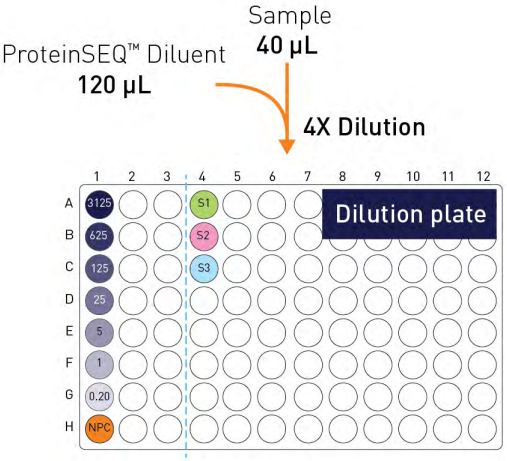

Figure 3 Dilute samples (example 4X dilution; shown in plate)

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Step 3: Prepare plates for the MagMAX™ Express-96 run

Label plates

1. Label 8 plates:

No. of plates	Plate type	Cat. no.	Label(s)
6	MagMAX™ Express-96 Skirted Low Profile Plates	4472783	Capture Probes Wash1 Wash 2 Wash 3 Wash 4
1	MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL) *or* MicroAmp® Optical 96-Well Reaction Plate with Barcode (0.2 mL)	4346906 *or* 4306737	qPCR
1	MagMAX™ Express-96 Standard Plate (200 µL)	4388475	Comb

2. Insert the plate labelled "qPCR" into a Fast 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 labelled "Comb".

Prepare wash plates

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

Prepare qPCR plate

Dispense 15 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	2850 µL	5700 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	3000 µL	6000 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 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.

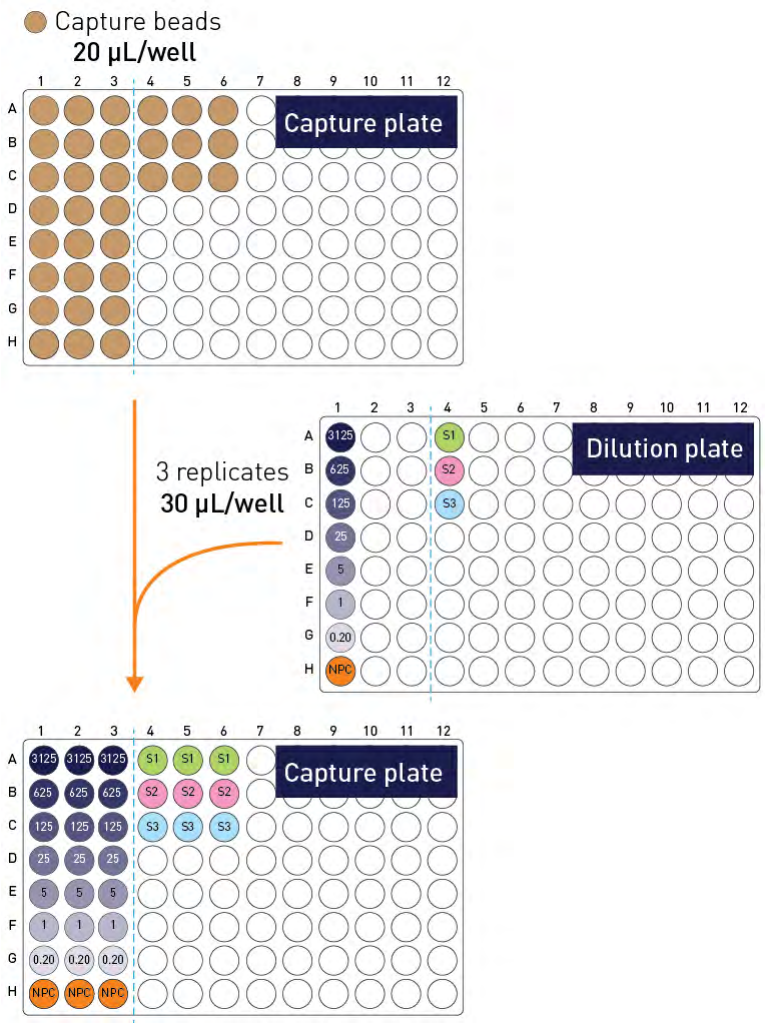

Figure 4 Transfer standards and samples to the capture plate

Step 4: Run plates in the MagMAX™ Express-96 Magnetic Particle Processor

1. Turn on the MagMAX™ Express-96 instrument and select the **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 MagMAX™ Express-96 turntable, starting with "Comb" (see Figure 5). Slide each plate into the plate hold-down (if present).

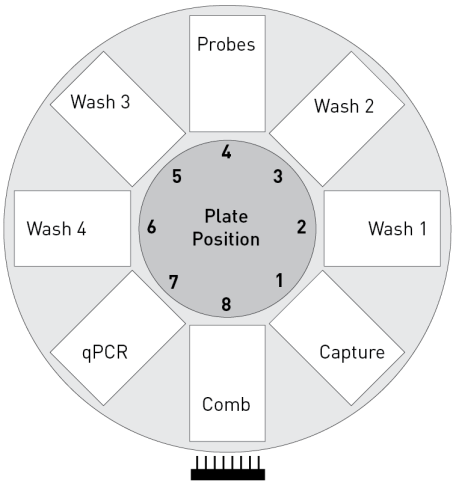

Figure 5 Plate positions in the MagMAX™ Express-96 turntable

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

   The run requires ~2 hours. When the run is complete, the screen displays "Proceed to qPCR".
4. When the MagMAX™ Express-96 program is complete, carefully remove the qPCR plate. Discard the Capture, Wash, and Probes plates.

Proceed immediately to "Step 5: Run qPCR reaction" on page 3.

Step 5: Run qPCR reaction

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

   Table 2 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	Green	2 µL	96 µL	192 µL
ProteinSEQ™ Ligase	Orange	0.20 µL	9.6 µL	19.2 µL
Total		22.2 µL	1065.5 µL	2132.2 µL

   [1] Includes 35% overage.
2. Transfer 15 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 Fast Real-Time PCR System (or equivalent).
4. Set up the run using AccuSEQ® system software (or equivalent, for example, SDS 1.4 software) as follows:
   -

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 **30 µL** with detection dye set to **FAM** and the quencher set to **none**.
5. Start the run.
6. 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.

Step 6: Perform data analysis

Perform data analysis with AccuSEQ® software

1. In the AccuSEQ® software, select autobaseline **on** and set the threshold manually to **0.2**.
2. Use the AccuSEQ® software to fit standards to a curve using a non-linear method and 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** and 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 HCP Master Template (a Microsoft® Excel® template available from your local sales or service representative) facilitates this process.
3. Fit standards to a curve using a non-linear method and 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

The information in this guide is subject to change without notice.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

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