resDNASEQ® Quantitative CHO DNA Kit

Catalog Numbers 4402085 (kit), 4442731 (kit with protocol and quick reference card), 4413713 (combo kit†), and 4415413 (combo kit with protocol and quick reference card)

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *resDNASEQ® Quantitative CHO DNA Kit User Guide* (Pub. no. 4415260). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product overview

The resDNASEQ® Quantitative CHO DNA Kit is used to quantitate host-cell residual DNA from Chinese hamster ovary (CHO) cells, a widely used cell line for production of biopharmaceutical products. Use the kit after you extract host-cell DNA from test samples. The resDNASEQ® Quantitative CHO DNA Kit uses TaqMan® quantitative PCR to perform rapid, specific quantitation of sub-picogram levels of residual CHO host-cell DNA. The assay is accurate and reliable across a broad range of sample types, from in-process samples to final product.

Kit contents and storage

Reagent	Description	Storage
resDNASEQ™ CHO DNA Control		
CHO DNA Control	1 tube, 40 µL	Store at –15 to –25°C.
DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –25°C before first use. Store at 2–8°C after first use.
resDNASEQ™ CHO Real-Time PCR Reagents		
2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –25°C before first use, protected from light. Store at 2–8°C after first use.
10X CHO DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25°C, protected from light.
Negative Control	1 tube, 1.0 mL	Store at –15 to –25°C before first use. Store at 2–8°C after first use.

Prepare for quantitative CHO DNA analysis

Guidelines

When you prepare the serial dilutions of CHO DNA, follow these rules to *avoid carryover contamination* and to ensure proper sample preparation and quantitative PCR (qPCR) of samples:

- Use Ambion® nonstick 1.5-mL tubes.
- Label the top of each tube for identification.
- Use pipettes for the serial dilutions and standard curve that are separate from those used for sample preparation or PCR set up.
- Prepare the serial dilutions in an area physically separate from the test-sample preparation area.

IMPORTANT! To assure accurate quantitative results, Life Technologies protocols call for true triplicate sample preparation and analysis. Extract each test sample in triplicate and perform a single PCR for each extraction. The instrument software then calculates a mean quantity and standard deviation for the triplicate samples, followed by a percent coefficient of variation from this data (SD/Mean Quantity × 100 = %CV). Based on the method qualification results, you can then assign a %CV to ensure accurate results from each sample tested.

† combo = PrepSEQ® Residual DNA Sample Preparation Kit + resDNASEQ® Quantitative CHO DNA Kit

Prepare CHO DNA serial dilutions

Prepare serial dilutions of CHO DNA control from the same experiment to create a standard curve and determine sample recovery rate.

To prepare the tubes for the CHO DNA standard curve, use the following table:

Standard curve (SC) tube	Volume of indicated serial dilution (SD) tube (µL)	Volume of PCR mix (µL)	Amount of DNA (pg)
SC1	SD1: 33	66	3000
SC2	SD2: 33	66	300
SC3	SD3: 33	66	30
SC4	SD4: 33	66	3
SC5	SD5: 33	66	0.3
SC6	SD6: 33	66	0.03

To prepare the reaction master mix, use the following table:

Kit Reagents	Volume for 1 30-µL reaction (µL)	Volume for 36 30-µL reactions (µL)
Negative Control	2	**72**
10X Primer/probe Mix	3	**108**
2X Environmental Master Mix	15	**540**
DNA Template	10	**NA**
Total	30	**720**

To set up a 96-well PCR reaction plate, use the following table:

	1	2	3	4	5	6	7	8	9	10	11	12
										Standard Curve (pg)		
A	NTC†	NTC	NTC							**3000**	**3000**	**3000**
B	NEG‡	NEG	NEG							**300**	**300**	**300**
C										**30**	**30**	**30**
D										**3**	**3**	**3**
E	TS§-1	TS-1	TS-1							**0.3**	**0.3**	**0.3**
F	TS-2	TS-2	TS-2							**0.03**	**0.03**	**0.03**
G	TS-3	TS-3	TS-3									
H												

† NTC = no template control
‡ NEG = negative control
§ TS = test sample

Workflow to prepare the serial dilutions and the standard curve

Prepare CHO DNA serial dilutions for the standard curve

Step 1: Label 7 Ambion® nonstick 1.5-mL tubes: **SD1**, **SD2**, **SD3**, **SD4**, **SD5**, **SD6**, and **NTC**.

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Step 2: Add 990 µL of DNA dilution Buffer (DDB) to tube SD1.

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Step 3: Add 450 µL of DDB to tubes SD2, SD3, SD4, SD5, SD6, and NTC.

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Step 4: Remove the tube of CHO DNA Control (30 ng/µL) from the freezer.

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Step 5: Thaw on ice, vortex gently for 2 sec, and quick-spin.

Step 6: Add 10 µL of CHO DNA Control to the tube that is labeled SD1, then vortex thoroughly.

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Step 7: Transfer 50 µL of the DNA from tube SD1 to tube SD2.

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Step 8: Transfer 50 µL of DNA from the previous dilution tube to the next dilution tube, until you add DNA to tube SD6. After each transfer, vortex thoroughly.

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Step 9: Store the CHO DNA dilution tube at 4°C for use on the day of preparation. Otherwise, store the tubes at –20°C and use within 1 week.

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Prepare the reaction master mix

Step 1: Determine the number of controls and test samples to quantify.

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Step 2: Thaw all kit reagents completely at room temperature.

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Step 3: Prepare a PCR mix.

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Add the master mix to the test and standard curve samples

Step 1: Prepare tubes for the CHO DNA standard curve.

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Step 2: Prepare the negative control samples (1 tube labeled NEG).

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Step 3: Prepare the test samples.

Step 4: Prepare the No Template Control samples (1 tube labeled NTC).

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Step 5: Set up a 96-well PCR plate.

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Step 6: Seal the plate with optical film, then quick-spin with a centrifuge rotor that is compatible with 96-well plates.

To run the preparation on the 7500 Fast instrument, go to the next page.

Create, run, and analyze a plate on the 7500 Fast instrument

To set up a plate layout, use the following plate example:

	1	2	3	4	5	6	7	8	9	10	11	12
										Standard Curve (pg)		
A	NTC	NTC	NTC							**3000**	**3000**	**3000**
B	NEG	NEG	NEG							**300**	**300**	**300**
C										**30**	**30**	**30**
D										**3**	**3**	**3**
E	TS-1	TS-1	TS-1							**0.3**	**0.3**	**0.3**
F	TS-2	TS-2	TS-2							**0.03**	**0.03**	**0.03**
G	TS-3	TS-3	TS-3									
H												

To set up the standard curve, use the following table:

Tube label	Row-wells	Task	Quantity	Label (pg)
SC1	A-10, 11, 12	Standard	3000	3000
SC2	B-10, 11, 12	Standard	300	300
SC3	C-10, 11, 12	Standard	30	30
SC4	D-10, 11, 12	Standard	3	3
SC5	E-10, 11, 12	Standard	0.3	0.3
SC6	F-10, 11, 12	Standard	0.03	0.03

To set up tests and controls, use the following table:

Tube label	Row-wells	Task	Quantity (pg)	Label
NTC	A-1, 2, 3	NTC	NA	NTC
NEG	B-1, 2, 3	NTC	NA	NEG
TS-1	E-1, 2, 3	Unknown	NA	Sample-1
TS-2	F-1, 2, 3	Unknown	NA	Sample-2
TS-3	G-1, 2, 3	Unknown	NA	Sample-3

To set the thermal cycling temperature and time, use the following table:

	AmpliTaq Gold® enzyme activation	PCR	
	Hold	40 Cycles	
		Denature	Anneal/extend
Temp (°C)	95	95	60
Time (min:sec)	10:00	0:15	1:00

Workflow to create the plate document, run the plate, and analyze the results

On the 7500 Fast instrument:

Create a plate document

Step 1: Select **Absolute Quantification** from the Template Assay drop-down list.

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Step 2: Select **Standard 7500** from the Run Mode drop-down list.

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Step 3: Enter **resDNAassay** in the Plate name field, then click **Next**.

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Step 4: Click **New Detector** and enter/select variables (name = **CHO**, report dye = **FAM**, quencher dye = **none**, select detector color), then click **Create Another**.

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Step 5: Click **New Detector** and enter/select variables (name = **IPC**, report dye = **VIC**, quencher dye = **none**, select detector color).

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Step 6: Select the applicable set of wells for the samples, then select FAM/IPC detectors for each well.

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Step 7: Set up the standard curve (select the wells, assign the tasks, and label the appropriate wells).

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Step 8: Set up the test and controls (select the wells, assign the tasks, and label the appropriate wells), then click **Finish**.

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Step 9: Select the **Setup** tab ▸ **Plate** tab, select and label the appropriate wells.

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Step 10: In the **Instrument** tab, set thermal-cycling conditions (reaction volume = **30 µL**, reaction = **standard**, set temperature and time).

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Step 11: Select **File** ▸ **Save as** and confirm that the file is named "resDNAassay", then select **SDS Templates (*.sdt)** in the "Save as type" drop-down list and close the plate document (template).

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Run the plate

Step 1: In the SDS software, select **File** ▸ **New**, then navigate to the SDS Documents folder.

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Step 2: Select the **resDNAassay** template file, then click **Open**.

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Step 3: In the Plate Name field, **enter ResDNA_*date of assay***, then click **Finish** in the New Document Wizard.

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Step 4: Make necessary changes to the test sample labels.

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Step 5: Load the plate on the instrument.

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Step 6: Select the **Instrument** tab, save the document, then click **Start** to start the real-time qPCR run.

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Analyze the results

Step 1: Select **Analysis** ▸ **Analysis Settings** from the **Results** tab.

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Step 2: In the Analysis Settings window:

- Select **Manual Ct**,
- Enter **0.2** in the Threshold field,
- Select **Manual Baseline**,
- Enter **3** for Start (cycle),
- Enter **15** for End (cycle),
- Click OK.

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Step 3: Click ▶ in the toolbar to analyze the plate.

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Step 4: Select the **Results** tab ▸ **Standard Curve** tab, then verify the Slope, Intercept, and R2 values.

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Step 5: Right-click the **Standard Curve**, select **Export as JPEG**, then click **OK**. Alternatively, press **PrintScreen**, then paste the image in a WordPad file.

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Step 6: Select **Report** tab ▸ **Report**, then review the mean quantity and standard deviation for each of the samples.

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Step 7: Select **File** ▸ **Export** ▸ **Results**. In the "Save as type" drop-down list, select **Results Export Files (*.csv)**, then click **Save**.

Limited product warranty

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Notes

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