Applied Biosystems

resDNASEQ™ Quantitative CHO DNA Kit

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *resDNASEQ™ Quantitative CHO DNA Kit Protocol* (PN 4415260). For all chemicals in **bold red** type, read the MSDS 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

The resDNASEQ™ Quantitative CHO DNA Kit components are shown in the table below.

Reagent	Description	Storage	Part number
resDNASEQ™ CHO DNA Control			**4403965**
CHO DNA Control	1 tube, 40 µL.	Store at –15 to –25 °C.	4403967
DNA Dilution Buffer (DDB)	1 bottle, 7 mL.	Store at –15 to –25 °C before first use. Store at 2 to 8 °C after first use.	4405587
resDNASEQ™ CHO Real-Time PCR Reagents			**4402431**
2× Environmental Master Mix	2 tubes, 0.75 mL/tube.	Store at –15 to –25 °C before first use, protected from light. Store at 2 to 8 °C after first use.	4401975
10× CHO DNA Real-Time PCR Assay Mix	1 tube, 300 µL.	Store at –15 to –25 °C, protected from light.	4402087
Negative Control	1 tube, 1.0 mL.	Store at –15 °to –25 °C before first use. Store at 2 to 8 °C after first use.	362250

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 separate from those that you use to prepare the sample preparation or to set PCR reactions.
- Prepare the serial dilutions in an area physically separate from the test-sample preparation area.

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

Prepare CHO DNA serial dilutions

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

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

Standard curve (SC) tube	Volume to transfer from indicated serial dilution (SD) tube (µL)	Volume of PCR reaction mix (µL)	Amt. of DNA (pg)
SC 1	(SD 1) 33	66	3000
SC 2	(SD 2) 33	66	300
SC 3	(SD 3) 33	66	30
SC 4	(SD 4) 33	66	3
SC 5	(SD 5) 33	66	0.3
SC 6	(SD 6) 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**
10 × primer/probe mix	3	**108**
2 × 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												

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**.

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

Step 3: Add 450 µL of DNA dilution buffer (DDB) to tubes SD2, SD3, SD4, SD5, SD6 and NTC.

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

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

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

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

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.

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

Prepare the reaction master mix

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

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

Step 3: Prepare a PCR reaction mix.

Add the master mix to the test and standard curve samples

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

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

Step 3: Prepare the test samples.

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

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

Step 6: Seal the plate with an 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)
SC 1	A-10, 11, 12	Standard	3000	3000
SC 2	B-10, 11, 12	Standard	300	300
SC 3	C-10, 11, 12	Standard	30	30
SC 4	D-10, 11, 12	Standard	3	3
SC 5	E-10, 11, 12	Standard	0.3	0.3
SC 6	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	D-1, 2, 3	Unknown	NA	Sample-1
TS-2	E-1, 2, 3	Unknown	NA	Sample-2
TS-3	F-1, 2, 3	Unknown	NA	Sample-3

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

Step	**AmpliTaq Gold® enzyme activation**	**PCR**	
	Hold	Cycle (40 Cycles)	
		Denature	Anneal/extend
Temp (°C)	95	95	60
Time (mm: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.

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

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

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

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

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

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

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

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

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

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).

Run the plate

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

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

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

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

Step 5: Load the plate on the instrument.

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

Analyze the results

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

Step 2: In the Analysis Settings window: select **Manual Ct**, in the Threshold field enter **0.2**, select **Manual Baseline** and enter **3** for Start (cycle) and **15** for End (cycle), then click **OK**.

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

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

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.

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

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

Notes

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