CHO Residual DNA Size Analysis Kit

Cat.No.: HYI161

Product Description

HYASEN CHO Residual DNA Size Analysis Kit is used to quantitate CHO DNA residues of different fragment sizes in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes fluorescent quantitative PCR technique (FAM) to perform rapid and specific quantitation of residual CHO DNA fragments in samples. The assay is designed to amplify four different fragments (95bp, 110bp, 215bp, 523bp) for the accurate size distribution, and the detection limit reaches femtograms (fg) level. For extraction information, please refer to the HYASEN Residual Host Cell DNA Sample Preparation Kit (Cat. No.: HYI126).

Components & Storage

Reagent	Quantity	Storage
qPCR Reaction Buffer	850 uL x 8 tubes	-20℃ protect form light
CHO Primer&Probe MIX-95	300 uL x 1 tube	
CHO Primer&Probe MIX-110	300 uL x 1 tube	
CHO Primer&Probe MIX-215	300 uL x 1 tube	
CHO Primer&Probe MIX-523	300 uL x 1 tube	
IPC MIX	550 uL x 1 tube	
CHO DNA Control	50 uL x 1 tube	-20℃
DNA Dilution Buffer (DDB)	1.5 mL × 3 tubes	

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

Applied instruments, including but not limited to the following

- 7500 Real-Time PCR System
- CFX96 Real-Time PCR System
- Lightcycler480 Real-Time PCR System

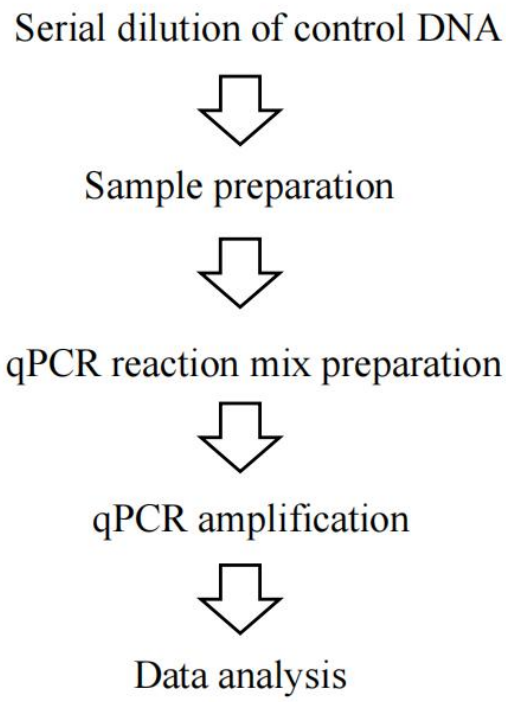
Required materials not included in the kit

- Nonstick, DNase-free&Low Retention Microfuge Tubes, 1.5mL
- Nonstick, Low Retention Tips, 1000μL, 100μL, 10μL
- 96-well qPCR plates or PCR 8-strip tubes

Related equipment

- Real-Time PCR System
- Vortex mixer
- Micro plate shaker
- Pipettes, 1000μL, 100μL, 10μL
- Microplate shaker

Workflow



Instructions

1. Experiment preparation

1) Wear appropriate protective eyewear, mask, clothing and gloves.
2) Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.
3) Thaw the kit completely at 2-8°C or melt on ice.

2. DNA Control serial dilutions for the standard curve

Note: The kit contains four CHO primer & probe mixes for different fragment lengths. Please set up four separate standard curves corresponding to each fragment length.

Please check the concentration on the label of the tube containing the CHO DNA Control prior to dilution.

Prepare CHO DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1) Thaw CHO DNA Control and DDB completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2) Label six nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
3) Dilute the DNA Control to 3000 pg/µL with DDB in the ST0 tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
4) Add 180 µL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.
5) Perform the serial dilutions according to Table 2:

Table 2. Dilution for CHO DNA Control

Serial dilution tube	Dilution	Conc. (pg/µL)
ST0	Dilute the DNA Control with DDB	3000
ST1	20 µL ST0 + 180 µL DDB	300
ST2	20 µL ST1 + 180 µL DDB	30
ST3	20 µL ST2 + 180 µL DDB	3
ST4	20 µL ST3 + 180 µL DDB	0.3
ST5	20 µL ST4 + 180 µL DDB	0.03

- *The remaining unused DDB need to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clears.*
- *At least five concentration of standard curve should be included. To select appropriate sample dilutions, we recommend performing method validation before sample testing.*

3. Sample preparation

1) Test Sample Preparation

Take 100 µL of the test sample and add it to a new 1.5 mL centrifuge tube.

2) Extraction Reference Control (ERC) samples Preparation

According to the CHO DNA spike concentration in ERC samples (Take the samples containing 30 pg of CHO DNA as example), the specific preparation procedure is as follows:

a. Take 100 µL of the test sample to a new 1.5 mL centrifuge tube.

b. Add 10 µL of ST3 solution and mix thoroughly, label it as the ERC sample.

3) Negative Control Sample (NCS) Preparation

Add 100 µL of DDB to a new 1.5 mL centrifuge tube, and label it as NCS.

4. qPCR MIX preparation

1) Determine the number of reaction wells based on the standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample. Number of reaction wells = (standard curve of 5 concentration gradients + 1 NTC + 1 NCS + test samples)×3

2) Prepare qPCR MIX seperately according to Table 3 - 6.

Table 3. qPCR MIX-95 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 µL	524.7 µL
CHO Primer&Probe MIX-95	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 4. qPCR MIX-110 Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 µL	524.7 µL
CHO Primer&Probe MIX-110	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 5. qPCR MIX-215 Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
CHO Primer&Probe MIX-215	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 6. qPCR MIX-523 Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
CHO Primer&Probe MIX-523	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

3) After thoroughly mixing qPCR MIX, follow 20 µL each tube is divided into PCR 8-strip tubes or 96-well qPCR plate.

5. qPCR Reaction MIX preparation

1) Prepare qPCR Reaction MIX according to Table 7-10 and 96-well plate layout as shown in Table 11.

Table 7. qPCR Reaction MIX-95 Preparation

Tubes	Standard curve-95	NTC	NCS	Test sample
qPCR MIX	20 µL	20 µL	20 µL	20 µL
Samples	10 µL ST1 - ST5	10 µL DDB	10 µL purified NCS	10 µL purified test sample
Total Volume	30 µL	30 µL	30 µL	30 µL

Table 8. qPCR Reaction MIX-110 Preparation

Tubes	Standard curve-110	NTC	NCS	Test sample
qPCR MIX	20 µL	20 µL	20 µL	20 µL
Samples	10 µL ST1 - ST5	10 µL DDB	10 µL purified NCS	10 µL purified test sample
Total Volume	30 µL	30 µL	30 µL	30 µL

Table 9. qPCR Reaction MIX-215 Preparation

Tubes	**Standard curve-215**	**NTC**	**NCS**	**Test sample**
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 10. qPCR Reaction MIX-523 Preparation

Tubes	**Standard curve-523**	**NTC**	**NCS**	**Test sample**
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 11. Example of 96-well Plate layout

Plate 1：

	MIX-95						MIX-110					
A	ST1	ST1	ST1	S1	S2	NTC	ST1	ST1	ST1	S1	S2	NTC
B	ST2	ST2	ST2	S1	S2	NTC	ST2	ST2	ST2	S1	S2	NTC
C	ST3	ST3	ST3	S1	S2	NTC	ST3	ST3	ST3	S1	S2	NTC
D	ST4	ST4	ST4				ST4	ST4	ST4			
E	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS
F				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
G				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
H												

Plate 2：

	MIX-215						MIX-523					
A	ST1	ST1	ST1	S1	S2	NTC	ST1	ST1	ST1	S1	S2	NTC
B	ST2	ST2	ST2	S1	S2	NTC	ST2	ST2	ST2	S1	S2	NTC
C	ST3	ST3	ST3	S1	S2	NTC	ST3	ST3	ST3	S1	S2	NTC
D	ST4	ST4	ST4				ST4	ST4	ST4			
E	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS
F				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
G				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
H												

- *This example represents four assays, including selected standard curve points of CHO DNA Control (ST1～ST5), 1 NTC, 1 NCS, 2 test samples(S1-S2) and 2 ERC samples (S1 ERC to S2 ERC), with 3 replicates for each sample.*
- *The plate layout for sample loading can be adjusted based on the sample Quantity*

2) Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in microcentrifuge and place it on the qPCR instrument.

6. qPCR program setting

NOTE: The following instructions apply only to the ABI7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.

1) Create a new document, then in the Assay drop-down list, select **Standard Curve (Absolute Quantitation)**.
2) Click **New Detector**, then enter CHO-95 in the Name field, select **FAM** in the Reporter Dye drop-down list and select (**none**) in the Quencher Dye drop-down list, then click **OK**.
3) Create new detector for CHO-110, CHO-215 and CHO-523, separately as step2.
4) Click **New Detector**, then enter IPC in the Name field. Select **VIC** in the Reporter Dye drop-down list and select (**none**) in the Quencher Dye drop-down list, then click **OK**.
5) Select **ROX** as the passive reference dye, then Click **Next**.
6) Select the applicable set of wells for the samples, then select the corresponding detector for each well.

7) Select Finish, and then set thermal-cycling conditions:

a. Set the thermal cycling reaction volume to 30 µL.

b. Set the temperature and time as Table 12:

Table 12. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing	60℃	00:30	
Extension	72℃*	01:30	

*Instrument will read the fluorescence signal during this step

8) Save the document, then click **Start** to start the real-time qPCR run.

7. Results analysis

1) Select **Set up** tab,then set tasks for each sample type by clicking on the Task Column drop-down list:

a. NTC: target DNA detector task = **NTC**

b. NCS, test samples = **Unknown**

2) Set up the standard curve as shown in the following table:

Table 13. Settings for Standard curve

Tube label	Task	Quantity (pg/µL)
ST1	Standard	300
ST2	Standard	30
ST3	Standard	3
ST4	Standard	0.3
ST5	Standard	0.03

3) Select the **Results tab**, then select Amplification Plot.

4) In the Data drop-down list, select **Delta Rn vs Cycle**.

5) In the Analysis Settings window, enter the following settings:

a. Select **Manual Ct**.

b. In the Threshold field, enter 0.02 .

c. Select **Automatic Baseline**.

6) Click the button ▶ in the toolbar, then wait the plate analyzing.

7) Select the **Result** tab>>**Standard curve** tab, then verify the Slope, Intercept and R^2 values.

8) Select the Report tab, then achieve the mean quantity and standard deviation for each sample.
9) Select **File** > > **Export** > > **Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
10) Set the value of DNA size-95 to be 100%, calculate the percentage of the DNA size of 110, 215 and 523.

Note: The parameter settings of the result analysis should be based on the specific model and the software version, and generally can also be automatically interpreted by the instrument.