HEK293 Residual DNA Size Analysis Kit

Cat.No.: HYI163

Product Description

HYASEN HEK293 Residual DNA Size Analysis Kit is used to quantitate HEK293 DNA residues of different fragment sizes for various stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes real-time PCR technology to perform rapid and specific quantitation of residual HEK293 DNA fragments in samples. It is designed to amplify four different fragments (75bp, 122bp, 244bp, 562bp) for the accurate determination of their size distribution at the femtogram (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
HEK293 Primer&Probe MIX-75	300 uL x 1 tube	
HEK293 Primer&Probe MIX-122	300 uL x 1 tube	
HEK293 Primer&Probe MIX-244	300 uL x 1 tube	
HEK293 Primer&Probe MIX-562	300 uL x 1 tube	
IPC MIX	550 uL x 1 tube	
HEK293 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

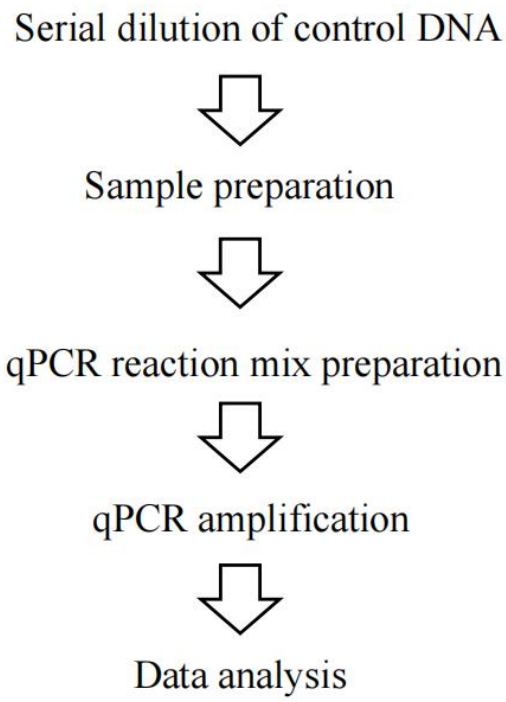
Required materials not included in the kit

- 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 HEK293 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 HEK293 DNA Control prior to dilution.

Prepare four sets of HEK293 DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1) Thaw HEK293 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 HEK293 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

Add 100 µL of DDB to a new 1.5 mL microfuge tube, and label as NCS. NCS and samples should be prepared in same way for DNA extraction.

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-75 preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
HEK293 Primer&Probe MIX-75	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 4. qPCR MIX-122 Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
HEK293 Primer&Probe MIX-122	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 5. qPCR MIX-244 Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
HEK293 Primer&Probe MIX-244	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

Table 6. qPCR MIX-562 Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 µL	524.7 µL
HEK293 Primer&Probe MIX-562	2.8 µL	92.4 µL
IPC MIX	1.3 µL	42.9 µL
Total volume	20 µL	660 µL

For simultaneous detection of the four fragments, please prepare at least 120 µL template DNA for four assays.

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-85 Preparation

ST-75	20 µL qPCR MIX-75 + 10 µL ST1/ST2/ST3/ST4/ ST5
NTC	20 µL qPCR MIX-75 + 10 µL DDB
NCS	20 µL qPCR MIX-75 + 10 µL purified NCS
Test sample	20 µL qPCR MIX-75 + 10 µL purified test sample

Table 8. qPCR Reaction MIX-122 Preparation

ST-122	20 µL qPCR MIX-122 + 10 µL ST1/ST2/ST3/ST4/ ST5
NTC	20 µL qPCR MIX-122 + 10 µL DDB
NCS	20 µL qPCR MIX-122 + 10 µL purified NCS
Test sample	20 µL qPCR MIX-122 + 10 µL purified test sample

Table 9. qPCR Reaction MIX-244 Preparation

ST-244	20 µL qPCR MIX-244 + 10 µL ST1/ST2/ST3/ST4/ ST5
NTC	20 µL qPCR MIX-244 + 10 µL DDB
NCS	20 µL qPCR MIX-244 + 10 µL purified NCS
Test sample	20 µL qPCR MIX-244 + 10 µL purified test sample

Table 10. qPCR Reaction MIX-562 Preparation

ST-562	20 μL qPCR MIX-562 + 10 μL ST1/ST2/ST3/ST4/ ST5
NTC	20 μL qPCR MIX-562 + 10 μL DDB
NCS	20 μL qPCR MIX-562 + 10 μL purified NCS
Test sample	20 μL qPCR MIX-562 + 10 μL purified test sample

Table 11. Example of 96-well Plate layout

	MIX-75			MIX-122			MIX-244			MIX-562		
A	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
B	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS
C	S	S	S	S	S	S	S	S	S	S	S	S
D	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5
E	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4
F	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3
G	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2
H	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1

- *This example represents four assays, including selected standard curve points of HEK293 DNA Control (ST1-ST5),1 NTC,1 NCS and 1 test sample, 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 HEK293-75 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 HEK293-122, HEK293-244 and HEK293-562, 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 DNA size of 75 to be 100%, calculate the percentage of the DNA size of 122, 244 and 562.
11) Analyze the Ct value of IPC. Normally, the Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, it indicates that the sample may be inhibited. It is recommended testing the ERC samples at the same time, and take the sample recovery rate result as the criterion.
12) The Ct value of NTC and NCS should be larger than the mean Ct value of the lowest concentration in the standard curve.

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.