# CHO Residual DNA Detection Kit

**Cat.No.: HYI122**

## Product Description

HYASEN CHO Residual DNA Detection Kit is used to quantitate host-cell line residual DNA from CHO cell line, which is used for production of biopharmaceutical products. This kit uses duplex real-time PCR technology to performs rapid, specific, and reliable quantitation assay at the fg level. IPC(Internal Positive Control) is included in the CHO Primer&Probe MIX to evaluate the performance of each PCR reaction. For extraction information,please refer to the HYASEN Residual Host Cell DNA Sample Preparation Kit (Cat. No.: HYI126).

## Components & Storage

| Reagent | Quantity | Storage |
|---|---|---|
| CHO DNA Control | 50μL × 1 tube | -20℃ |
| qPCR Master MIX | 850 μL × 2 tubes | -20℃<br>protect form light |
| DNA Dilution Buffer (DDB) | 1.5 mL × 3 tubes | -20℃ |
| CHO Primer&Probe MIX<br>(Incl IPC) | 500 μL × 1 tube | -20℃<br>protect form light |

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

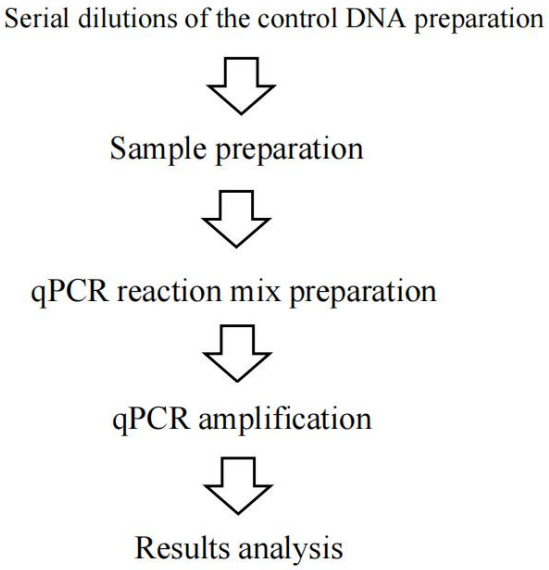
## Required materials not included in the kit

- 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

## 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, vortex and spin briefly.

### 2. DNA Control serial dilutions for the standard curve

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

1) Thaw CHO DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5seconds in microcentrifuge, and repeat 3 times.
2) Label seven nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4, ST5, ST6.

3) Dilute the CHO 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 it thoroughly.

4) Add 90 μL DDB to each tube: ST1, ST2, ST3, ST4, ST5, ST6.

5) Perform the serial dilutions:

Table 2. Dilution for CHO DNA Control

| Serial dilution tube | Dilution | Conc. (pg/μL) |
|---|---|---|
| ST0 | Dilute the DNA Control with DDB | 3000 |
| ST1 | 10 μL ST0 + 90 μL DDB | 300 |
| ST2 | 10 μL ST1 + 90 μL DDB | 30 |
| ST3 | 10 μL ST2 + 90 μL DDB | 3 |
| ST4 | 10 μL ST3 + 90 μL DDB | 0.3 |
| ST5 | 10 μL ST4 + 90 μL DDB | 0.03 |
| ST6 | 10 μL ST5 + 90 μL DDB | 0.003 |

- *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 = (6 standard points on the standard curve + 1 NTC + 1 NCS + test samples)×3

2) Prepare qPCR MIX according to the number of reaction wells.

Table 3. qPCR MIX Preparation

| Reagents | Volume/reaction | Volume for 30 reaction (includes 10% overage) |
|---|---|---|
| qPCR Master MIX | 15 µL | 495 µL |
| CHO Primer&Probe MIX (Incl IPC) | 5 µL | 165 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 4 and 96-well plate layout as shown in Table 5.

Table 4. qPCR Reaction MIX Preparation

| | |
|---|---|
| Standard curve | 20 µL qPCR MIX + 10 µL ST1/ST2/ST3/ST4/ ST5/ST6 |
| NTC | 20 µL qPCR MIX + 10 µL DDB |
| NCS | 20 µL qPCR MIX + 10 µL purified NCS |
| Test sample | 20 µL qPCR MIX + 10 µL purified test sample |
| Test sample ERC | 20 µL qPCR MIX + 10 µL purified ERC sample |

Table 5. Example of 96-well Plate layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NTC | | S1 | S1 | S1 | S1 ERC | S1 ERC | S1 ERC | | ST6 | ST6 | ST6 |
| B | NTC | | S2 | S2 | S2 | S2 ERC | S2 ERC | S2 ERC | | ST5 | ST5 | ST5 |
| C | NTC | | S3 | S3 | S3 | S3 ERC | S3 ERC | S3 ERC | | ST4 | ST4 | ST4 |
| D | | | S4 | S4 | S4 | S4 ERC | S4 ERC | S4 ERC | | ST3 | ST3 | ST3 |
| E | NCS | | S5 | S5 | S5 | S5 ERC | S5 ERC | S5 ERC | | ST2 | ST2 | ST2 |
| F | NCS | | | | | | | | | ST1 | ST1 | ST1 |
| G | NCS | | | | | | | | | | | |
| H | | | | | | | | | | | | |

- *This example represents the assay for a standard curve with 6 concentration gradients (ST1 to ST6), 1 NTC, 1 NCS, 5 test samples (S1 to S5), 5 ERC samples (S1 ERC to S5 ERC), and 3 replicates for each sample.*

- *In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity. Please refer to the example shown in Table 5.*

2) Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it in 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) In the Run Mode drop-down list, select **Standard 7500**, then click **Next.**
3) Click **New Detector:**

a. Enter CHO-DNA in the Name field.
b. Select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
c. Select a color for the detector, then click **Create Another**.

4) Click New Detector:

a. Enter IPC in the Name field.
b. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
c. Select a color for the detector, then click **OK**.
d. Select the detectors, then click **Add** to add the detectors to the document.

5) Select **ROX** as the passive reference dye, then Click **Next**.
6) Select the applicable set of wells for the samples, then select CHO-DNA detector and IPC 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 the time as following:

| Step | Temp. | Time(mm:sec) | Cycles |
|---|---|---|---|
| Activation | 95℃ | 10:00 | 1 |
| Denaturation | 95℃ | 00:15 | 40 |
| Annealing/extension | 60℃* | 1:00 | |

*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, and ERC wells: target DNA detector task = **Unknown**

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

Table 6. 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 |
| ST5 | Standard | 0.003 |

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, CHO-DNA enter 0.05 and IPC enter 0.1.

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) In the Report panel of Results, the 'Mean Quantity' column can read the detection values of NTC, NCS, test sample, and ERC sample, in pg/μL.

11) The recovery rate of ERC samples should be calculated based on the test results of the test samples and the ERC samples. The recovery rates should be between 50% and 150%.

12) The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve, and it shows normal amplification curve in the VIC signal channel.

13) The Ct value of NTC should be ≥ 35.00, or set specific standards based on the laboratory's own validation results, and it shows normal amplification curve in the VIC signal channel.

*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.*