# *E.coli* Residual RNA Detection Kit

**Cat.No.: HYI172**

## Product Description

HYASEN Residual *E. coli* RNA Quantitation Kit is used for quantitation of host cell RNA from *E. coli* in a variety of biopharmaceutical products. This kit employs reverse transcription quantitative PCR technique to performs a rapid, specific, and reliable quantitative detection of residual *E. coli* RNA.

## Components & Storage

| Reagent | Quantity | Storage |
|---|---|---|
| *E. coli* RNA Control | 50 µL × 1 tube | -20℃ |
| One Step qPCR Buffer | 500 µL × 2 tubes | -20℃<br>protect form light |
| One Step Enzyme MIX | 100 µL × 1 tube | -20℃<br>protect form light |
| *E. coli* RNA Primer&Probe MIX | 400 µL × 1 tube | -20℃<br>protect form light |
| RNA IPC Primer&Probe MIX | 200 µL × 1 tube | -20℃<br>protect form light |
| RNase-Free $H_2O$ | 1.2 mL × 3 tubes | -20℃ |

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
- Lightcycler 480 II Real-Time PCR System
- CFX96 Real-Time PCR System
- LineGene9600plus Real-Time PCR System

## Required materials not included in the kit

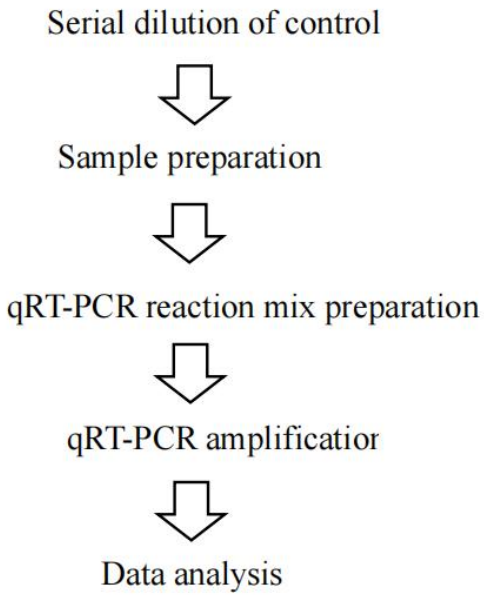
- Nonstick, RNase-free, Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips 1000 µL, 100 µL, 10 µL

- DNase I and Buffer
- RNase inhibitor (Optional)
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
- HYASEN Residual Host Cell RNA Sample Preparation Kit (Cat.No.: HYI171, Optional)

## Related equipment

- Mini Centrifuge
- Vortex mixer
- Real-Time PCR System
- Pipettes: 1000 µL, 100 µL and 10 µL
- Dry bath incubator
- Microplate shaker
- Real-time PCR System

## Workflow




## Instructions

### 1. Experiment preparation

1) Wear appropriate protective eyewear, mask, clothing and gloves.
2) Irradiate the tabletop, micropipettes 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. RNA Control serial dilutions for the standard curve

Please check the concentration on the label of *E. coli* RNA Control tube prior to dilution.

1) Thaw *E. coli* RNA Control and RNase-Free $H_2O$ completely at 2-8°C or melt on ice. Flick the *E. coli* RNA Control tube gently, and briefly centrifuge 3-5 s, and repeat 3 times to mix well.
2) Label seven nonstick 1.5mL microfuge tubes: A, B, ST1, ST2, ST3, ST4 and ST5, respectively.
3) Transfer certain amount of RNase-Free $H_2O$ and *E. coli* RNA Control to A tube to achieve a 2000 pg/μL control solution. Vortex and centrifuge briefly, and repeat for 3 times.
4) Add 45 μL RNase-Free $H_2O$ to each tube of B, ST1, ST2, ST3, ST4 and ST5. 5. Perform the serial dilutions according to Table 2:

   a. Transfer 5 μL of RNA from tube A to B, then vortex for 5 seconds and spin for 5 seconds. Repeat 3 times to mix thoroughly.

   b. Continue to transfer 5 μL of RNA to the next dilution tube until ST5.

Table 2. Dilution for *E. coli* RNA Control

| Serial dilution tube | Dilution | Conc. (pg/μL) |
|---|---|---|
| A | Dilute the RNA Control with RNase-Free $H_2O$ | 2000 |
| B | 5 μL A + 45 μL RNase-Free $H_2O$ | 200 |
| ST1 | 5 μL B + 45 μL RNase-Free $H_2O$ | 20 |
| ST2 | 5 μL ST1 + 45 μL RNase-Free $H_2O$ | 2 |
| ST3 | 5 μL ST2 + 45 μL RNase-Free $H_2O$ | 0.2 |
| ST4 | 5 μL ST3 + 45 μL RNase-Free $H_2O$ | 0.02 |
| ST5 | 5 μL ST4 + 45 μL RNase-Free $H_2O$ | 0.002 |

- *The remaining unused RNase-Free $H_2O$ can be stored at 2-8℃. If not used for a long time, please store at -20℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clear.*

- *It is recommended to include at least five concentration points for the standard curve, which can be selected based on the method validation data.*

## 3. Sample preparation

According to different sample types, two different preparation ways are provided as below:

➢ Plasmid DNA samples derived from *E. coli* host bacteria

1) Apply DNase I treatment to the test samples, ERC, and NCS to eliminate the interference of genomic DNA (gDNA) on the detection. Follow the DNase digestion method outlined in Table 3:

Table 3. DNase Digestion of samples

| **Reagents to add in one sample** | **Quantity required for each sample** | | | |
|---|---|---|---|---|
| | **NCS (µL)** (Choose option A or B)** | | **Sample (µL)** | **ERC Sample (µL)** |
| | **Option A: RNase-Free $H_2O$** | **Option B: Sample matrix** | | |
| 10×DNase I Buffer | 4 | 4 | 4 | 4 |
| DNase I (5 U/µL)*** | 4 | 4 | 4 | 4 |
| Sample or Sample matrix | 0 | 4 (Sample matrix) | 4*** (no more than 1mg/uL) | 4 |
| *E. coli* RNA Control | 0 | 0 | 0 | 10* |
| RNase inhibitor(Optional）**** | final concentration 0.2-1U/µL | | | |
| Add RNase-Free $H_2O$ to | 40uL | | | |
| Incubate each sample at 37℃ for 30 - 60 minutes for digestion. (The digestion conditions are determined based on actual experience) | | | | |

*Sample extraction recovery control (ERC): In principle, the ERC Sample should ideally have an actual *E. coli* RNA concentration that is 2-10 times higher than the detected value in the sample. If the *E. coli* RNA concentration in the sample is lower than the LOQ of this kit, the spiked quantity should be adjusted within the assay range to ensure the accurate results.

**You can choose either sample matrix or RNase-Free $H_2O$ for NCS.

***Ensure that the final concentration of plasmid sample does not exceed 100 ng/uL, which requires the final concentration of DNase I at 0.2-2U/µL.

****Add RNase inhibitor to the digestion reaction can help to mitigate the potential impact of RNase from samples, consumables, environment, etc.

2) Choose an appropriate DNase I inactivation method (one out of three methods below):

**Method 1:** Use HYASEN Residual Host Cell RNA Sample Preparation Kit to treat the digestion solution of test samples, samples ERC, and NCS. This inactivation method can effectively purify the sample matrix or digestion reaction solution, eliminating any potential matrix effects.

**Method 2:** Inactivate DNase I at 75℃ for 10 min.

**Method 3:** Use some other validated DNase inactivation or removal methods.

- Samples from protein expression products using *E. coli* host bacteria

1) Sample RNA Extraction

   Use HYASEN Residual Host Cell RNA Sample Preparation Kit to purify the test samples, ERC, and NCS.

2) Digest the extracted RNA samples from test samples, ERC sample, and NCS using DNase I to eliminate the potential influence of gDNA on the detection. Treat the digestion reactions according to Table 3.

3) Inactivate DNase I by heating the sample at 75℃ for 10 min.

## 4. qRT-PCR MIX preparation

1) Prepare qRT-PCR MIX according to Table 4:

Table 4. qRT-PCR MIX Preparation

| **Reagents** | **Volume/well** | **Volume for 48 reactions (includes 10% overage)** |
|---|---|---|
| One Step qPCR Buffer | 10 μL | 530 μL |
| One Step Enzyme MIX | 1 μL | 53 μL |
| *E. coli* RNA Primer&Probe MIX | 4 μL | 212 μL |
| Total | 15 μL | 795 μL |

2) Votex to mix well, and add 15 μL/well of qRT-PCR MIX to 96-well qPCR plates or 8-strip PCR tubes.

## 5. qRT-PCR Reaction MIX preparation

1) Prepare qRT-PCR Reaction MIX as shown in Table 5:

Table 5. qRT-PCR Reaction MIX preparation

| **Reagents** | **Standard curve** | **NTC** | **NCS** | **Test sample** | **ERC sample** |
|---|---|---|---|---|---|
| qRT-PCR MIX | 15 μL | 15 μL | 15 μL | 15 μL | 15 μL |
| Sample | 5 μL ST1/ST2/ST3/ ST4/ ST5 | 5 μL RNase-Free $H_2O$ | 5 μL purified NCS | 5 μL test sample | 5 μL purified ERC Sample |
| Total Volume | 20 μL | 20 μL | 20 μL | 20 μL | 20 μL |

## 6. IPC qRT-PCR Reaction MIX preparation

Note: If spiked samples are tested, the IPC group are considered as optional.

For IPC group, each experiment requires IPC-NCS as negative quality control and IPC-S for each test sample, and prepare the corresponding qRT-PCR MIX and qRT-PCR reaction mix according to Tables 6 and 7:

Table 6. IPC qRT-PCR MIX preparation

| Reagents | Volume/reaction | Volume for 30 reactions (includes 10% overage) |
|---|---|---|
| One Step qPCR Buffer | 10 μL | 330 μL |
| One Step Enzyme MIX | 1 μL | 33 μL |
| RNA IPC Primer&Probe MIX | 4 μL | 132 μL |
| Total | 15 μL | 495 μL |

Table 7. IPC qRT-PCR Reaction MIX preparation

| Reagents | IPC-NCS | IPC-S |
|---|---|---|
| IPC qRT-PCR MIX | 15 μL | 15 μL |
| Sample | 5 μL purified NCS | 5 μL test sample |
| Total Volume | 20 μL | 20 μL |

## 7. qPCR plate preparation

1) For the layout of the 96-well PCR plates, refer to Table 8.

Table 8. Plate layout of 96-well PCR reaction for example

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

- *This example represents the assay for a standard curve with 5 concentration gradients (ST1 to ST5), 1 no template control (NTC), 1 negative control sample (NCS), and 3 unknown test samples (S1 to S3), and 3 ERC samples (S1 ERC to S3 ERC), For IPC, Negative quality control (IPC-NCS) and test samples (IPC-S1, IPC-S2, IPC-S3). and 3 replicates are recommended 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 8.*

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

## 8. RT-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 *E. coli*-RNA 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 E.coli-RNA detector and IPC detector for Corresponding well.
7) Select Finish, and then set thermal-cycling conditions:

a. Set the thermal cycling reaction volume to 20 μL.
b. Set the RT-qPCR program as following:

Table 9. qPCR running temperature and time

| Step | Temp. | Time(mm:sec) | Cycles |
|---|---|---|---|
| Reverse transcription | 50 ℃ | 15 : 00 | 1 |
| Activation | 95℃ | 00 : 30 | 1 |
| Denaturation | 95℃ | 00 : 10 | 45 |
| Annealing/extension | 60℃* | 00 : 40 | |

*Instrument will read the fluorescence signal during this step

8) Save the document, then click Start to start the qRT-PCR run.

## 9. 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 RNA detector task = **NTC**

b. NCS, test samples, and ERC wells: target RNA detector task = **Unknown**

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

Table 10. Settings for Standard curve

| Tube label | Task | Conc. (pg/μL) |
|---|---|---|
| ST1 | Standard | 20 |
| ST2 | Standard | 2 |
| ST3 | Standard | 0.2 |
| ST4 | Standard | 0.02 |
| ST5 | Standard | 0.002 |

3) 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.

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

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

6) Select the Report tab, then achieve the mean quantity and standard deviation for each sample.

7) Select **File** > > **Export** > > **Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.

8) 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.

9) The average Ct value of NTC should be at least 2 cycles higher than the lowest standard curve.

10) 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%.
11) If the Ct -IPC value of the sample is significantly larger than the Ct-IPC value of NTC or NCS, it indicates that the reaction may be inhibited by test sample. Please consider sample recovery rate prior to IPC results, and IPC results can only be used as reference.

*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.*