CYGNUS TECHNOLOGIES
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Product Manual: D1415

E. coli AccuRes™ Quantitative DNA Kit

Contents

Intended Use

This qPCR kit is intended for use in determining the presence of host cell DNA contamination in products manufactured by recombinant expression in *E. coli*. The starting materials for this kit are samples that have already been subjected to DNA extraction (such as with Cygnus Cat# D100T or D100W). The kit contains calibrated DNA concentrate, primers and probe mix, and 2x PCR Master Mix for DNA amplification.

The kit is for **Research, Development and Manufacturing Use Only** and is not intended for diagnostic use in humans or animals.

Summary & Explanation

Expression of therapeutic proteins in *E. coli* is a cost-effective method for production of commercial quantities of a drug substance. However, the manufacturing and purification process of these products leaves the potential for residual DNA contamination from the host cells. Due to the theoretical potential for the transfer of oncogenes from the host cell, both the WHO and FDA have set the allowable limit as 10-100ng/dose, depending on the therapeutic regimen.

This kit is designed to measure *E. coli* host cell residual DNA for the purpose of process development, in-process monitoring, and QC lot release testing. Quantitative PCR (qPCR) based assays have been employed by many biopharmaceutical manufacturers over the years. However, in many cases proteins and buffer components present in the samples can interfere with DNA amplification, resulting in either over or under estimation of the true DNA concentration. Samples must be subjected to a DNA extraction procedure (such as with Cygnus Cat# D100T or D100W) to remove such interfering components prior to the use of this kit. This kit uses a proprietary qPCR master mix with hot start reagents and specific qPCR probes to ensure sensitive and accurate measurements of residual host cell DNA.

Principle of Procedure

This convenient, easy-to-use kit is compatible with your existing qPCR instruments. The kit includes DNA concentrate for preparation of standards, a cell-line specific AccuRes™ *E. coli* PCR Primers and Probe Mix, 10X, and AccuRes™ PCR Master Mix, 2X. Upon completion of extraction of residual DNA using your preferred method, the sample, standards, and controls are transferred to a qPCR plate with AccuRes™ Primers and Probe Mix and PCR Master Mix. After sealing the plate with the supplied optical seal, the qPCR plate is subjected to 40 amplification cycles. A standard curve is constructed from the point at which each standard crosses a pre-established threshold. The samples and controls are measured against the standard curve to determine the concentration of DNA. Using this method, residual DNA can be measured to a limit of detection (LOD) of 0.7 femtogram per microliter levels.

Reagents & Materials Provided

Component	Product #
***E. coli* DNA Concentrate,** 100ng/10µL x 120µL	D418
DNA TE Buffer, 30 mL	D001*
AccuRes™ PCR Master Mix, 2X, 2 x 750µL	D1005
AccuRes™ *E. coli* PCR Primers and Probe Mix, 10X, 1 x 350µL	D1417
AccuRes™ Deionized Water, 2 x 500µL	D1006
PCR Assay Plate with Optical Seal, 1 x 96 wells	D1004

*Component can be purchased separately.

Storage & Stability

The AccuRes™ *E. coli* PCR Primers and Probe Mix, 10X and AccuRes™ PCR Master Mix, 2X should be stored at -20°C for long-term storage. After thawing, The AccuRes™ *E. coli* PCR Primers and Probe Mix, 10X and AccuRes™ PCR Master Mix, 2X can be stored at 2°C to 8°C for stability until the expiration date printed on the kit label. If you are storing DNA samples for testing/retesting, we recommend short-term storage at 2-8˚C.

Materials & Equipment Required but Not Provided

- Uracil-DNA Glycosylase (NEB M0280L) for amplicon contamination control (if desired).
- Pipettors – 5µL – 1200µL.
- Real-time PCR instrument capable of detecting FAM signal

Precautions

- For Research, Development or Manufacturing use only.
- This kit should only be used by qualified technicians.

Preparation of Reagents

The standards and PCR plate can be prepared at room temperature.

Preparation of Standards

Prepare the Standard Curve by making 10-fold dilutions of the DNA Concentrate according to the table. Remove and discard 50µL from tube 8 and ensure that the final volume in each tube is 450µL. Discard tubes 1-3.

Tube #	*E. coli* DNA	TE Buffer	Final Concentration
1	Stock	N/A	100 ng/10 µL
2	50 µL of Tube 1	450 µL	10 ng/10 µL
3	50 µL of Tube 2	450 µL	1 ng/10 µL
4	50 µL of Tube 3	450 µL	100 pg/10 µL
5	50 µL of Tube 4	450 µL	10 pg/10 µL
6	50 µL of Tube 5	450 µL	1 pg/10 µL
7	50 µL of Tube 6	450 µL	0.1 pg/10 µL
8	50 µL of Tube 7	450 µL	0.01 pg/10 µL
9	0 µL	450 µL	0 pg/10 µL

Preparation of Samples

If the extracted DNA sample contains more than 10 mg/mL of protein, we recommend diluting the sample in TE buffer.

Amplification Reagent Preparation

Prepare the amplification reagent mixture using the below volumes per well for the qPCR assay, adding ~10% overage to accommodate for pipetting error.

Reagent	Volume/well
AccuRes™ PCR Master Mix (2X)	**15 µL**
AccuRes™ Primers and Probe Mix (10X)	**3 µL**
AccuRes™ Deionized Water	**2 µL**

Procedural Notes

1. Protein in the sample is a known interference factor in DNA quantification methods. DNA samples and controls must be extracted prior to testing in this kit. This can be done using commercially available kits from Cygnus (D100T or D100W) or another trusted vendor.
2. Due to the extreme sensitivity of this assay, it is important to keep the working area clean to avoid contamination by DNA in the environment. Thoroughly clean pipettes and the immediate working area prior to initiating the procedure. Remove anything from the area that is not required for the procedure. Avoid leaning over the samples as much as possible. Organize solutions and tips such that passing over the plate is minimized.

3. The CleanAmp® dNTP mix contained in AccuRes™ PCR Master Mix, 2X includes CleanAmp® dUTP which provides the opportunity to treat the reaction with UNG/UDG (not included) to eliminate contamination with other amplicons.
4. The DNA probe contained in the AccuRes™ Primers and Probe Mix is conjugated to a fluorophore (FAM) and Black Hole Quencher™ (BHQ™). During PCR extension, the probe is cleaved from the quencher, allowing for the release of quantifiable fluorescent signal.
5. ROX reference dye is included in the AccuRes™ PCR Master Mix to ensure normalization across sample wells during PCR amplification.
6. We recommend testing each sample and control in triplicate to ensure detection and quantification is as sensitive and robust as possible.

Limitations

This kit is designed for qPCR amplification of host cell DNA from samples subjected to DNA extraction by your preferred method (such as with Cygnus Cat# D100T or D100W).

Calculation of Results

The C_T values of the standards are used to construct a standard curve with values reported by the instrument in pg/10µL host cell residual DNA. The concentration of host cell residual DNA can be mathematically transformed for reporting residual DNA in ng/mL, ng/mg of drug product or in ng/dose.

DNA Amplification Protocol

1. **The amplification reagent is prepared by combining Cat# D1417 AccuRes™ *E. coli* Primers and Probe Mix, 10X (3 µL/well), Cat# D1005 AccuRes™ PCR Master Mix, 2X (15 µL/well), and Cat# D1006 AccuRes™ Deionized Water (2µL/well) as described in the 'Amplification Reagent Preparation' section.**
2. **Prepare 20µL of the amplification reagent for each well plus an additional 10% excess. For example, 96 wells + 10% = enough amplification reagent for 105 wells.**
3. **Transfer 20µL of amplification reagent to each well of the qPCR assay plate (Cat# D1004).**
4. **Transfer 10µL of each standard, test samples and controls to the qPCR assay plate. We recommend testing each sample in triplicate.**
5. **Apply the optical seal over the wells.**
6. **Gently tap the side of the plate to remove all bubbles from the bottom of the wells.**
7. **Place the assay plate into the qPCR instrument.**
8. **Suggested amplification parameters:**

   Step 1: 37°C 2 minutes (optional)

   Step 2: 95°C for 10 minutes.

   Step 3: 95°C for 15 seconds followed by 60°C for 1 minute (x 40 cycles).

Performance Characteristics

Cygnus Technologies has qualified this assay by conventional criteria as indicated below. A more detailed copy of this "Qualification Summary" report can be obtained by request. This qualification is generic in nature and is intended to supplement but not replace certain user and product specific qualification and validation that should be performed by each laboratory. At a minimum each laboratory is urged to perform a spike and recovery study for each sample type to be tested in the assay. Each laboratory technician should also demonstrate competency in the assay by performing a similar precision study to that described below. A more detailed discussion of recommended user validation protocols can be obtained by contacting our Technical Services Department or at our web site.

qPCR Amplicon

The AccuRes™ *E. coli* PCR Primers and Probe Mix provided in this kit will amplify a multi-copy ribosomal gene specific to *E. coli* DNA, producing an amplicon of 62 base pairs.

Accuracy and Precision

E. coli DNA samples were prepared in a 1 mg/mL human IgG sample matrix at various concentrations spanning the Standard Curve. Three preparations were made for each sample following DNA extraction and duplicate wells were collected for each preparation resulting in 12 individual results per concentration. The assays were performed over 2 days.

Concentration	% Nominal	Intra-assay CV	Inter-assay CV
50pg/10µL	87.4%	2.4%	10.2%
5pg/10µL	90.5%	4.3%	3.9%
0.5pg/10µL	92.0%	2.4%	3.6%
0.05pg/10µL	93.3%	4.3%	5.1%

Ordering Information/ Customer Service

To place an order or to obtain additional product information, contact:

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Need a Custom DNA Assay developed? - Contact Cygnus Experts

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