E. coli Host Cell DNA Extraction & Amplification Kit in Tubes

DNA Extraction and Amplification Kit for the Measurement of Residual *E. coli* Host Cell DNA
Catalog # D415T

Intended Use

This kit is intended for use in determining the presence of host cell DNA contamination in products manufactured by recombinant expression in *E. coli* cell lines. This kit contains reagents for DNA extraction as well as calibrated DNA concentrate and primers for DNA amplification. The kit does not contain the PCR master mix. PCR master mix must be purchased separately from Life Technologies Corporation or other licensed vendor.

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

Summary and Explanation

Expression of therapeutic proteins in *E. coli* cells 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 DNA contamination from the host cells. Due to the theoretical potential for the transfer of oncogenes from the host cell, the WHO has set a residual host cell DNA limit of 10ng/dose. Regulatory agencies have set allowable limits between 100pg/dose and 10ng/dose depending on the cell line used as well as the mode and frequency of dosing.

This kit is designed to measure residual host cell DNA for the purpose of process development, in-process monitoring, lot release and QC testing. Quantitative PCR (qPCR) based assays have been employed by many biopharmaceutical manufacturers over the years. However, in many cases proteins and buffer components can interfere with DNA amplification resulting in either over or under estimation of the true DNA concentration. This kit uses a proprietary DNA extraction procedure to isolate the residual DNA from complex matrices and perform the measurements in an environment free from contaminating proteins, salts and detergents. The removal of contaminating product protein and other excipients ensures accurate measurements of residual host cell DNA and allows for timely and scientifically sound process decisions.

Principle of the Procedure

This convenient, easy-to-use kit is compatible with your existing qPCR reagents and instruments. In addition to all materials required for Cygnus' proprietary extraction of DNA to remove PCR interfering components, the kit includes DNA concentrate for preparation of standards and a validated cell-line specific primer set. Upon completion of extraction of residual DNA, the sample, standards, and controls are transferred to a qPCR plate containing a user supplied master mix to which the Cygnus primer mix has been added. After sealing the plate with the supplied optical seal, the qPCR plate is subjected to 35–40 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 1 part per billion levels.

Reagents & Materials Provided

Component	Product #
DNA Extraction:	
Proteinase K, 1 x 150µL	**D101**
DNA Extraction Buffer, 1 x 30mL	**D108**
DNA Precipitation Buffer, 1 x 55mL	**D106**
DNA Wash Buffer, 1 x 100mL	**D103**
DNA Sample Diluent, 1 x 30mL	**D006***
2mL Sterile Microfuge Tubes, 50 tubes	**D107***
DNA Amplification:	
***E. coli* DNA Concentrate,** 100ng/10µL x 120µL	**D418**
***E. coli* Forward and Reverse Primer Mix,** 350µL	**D417**
PCR Assay Plate with Optical Seal, 1 x 96 wells	**D004***

*Component can be purchased separately.

Storage & Stability

All reagents should be stored at 2°C to 8°C for stability until the expiration date printed on the kit label.

Materials & Equipment Required But Not Provided

- qPCR master mix (Life Technologies Corp. or other licensed vendor)
- DNA TE Buffer, Cat# D001 (Tris/EDTA used for Proteinase K dilution and final pellet reconstitution)
- Pipettors – 5µL – 1200µL.
- Bench top microfuge capable of spinning 2mL tubes at 10,000 rpm.
- Dry heat block for use with tubes.
- Vortex
- Spray bottle
- Absorbent wipes

Precautions

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

Preparation of Reagents

Bring all reagents to room temperature prior to starting the extraction procedure.

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

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

Procedural Notes

1. Protein in the sample is a known interference factor in DNA quantification methods. Use of the proprietary Cygnus DNA Sample Diluent (Cat# D006) will generally allow for acceptable DNA recovery in up to 20mg/mL of protein. Samples with higher protein concentrations can be used but must be qualified in the assay.

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.

3. The Proteinase K digestion should be carried out at 60°C for most therapeutic proteins. Monoclonal antibodies generally perform well at 60°C. However, each laboratory may need to determine the optimum temperature for non-IgG drug products. Do not exceed 60°C as this may cause the protein to precipitate from solution.

4. Always make sure the centrifuge is balanced to ensure proper assay performance. An improperly balanced centrifuge can result in loose pellets, which can adversely affect recovery and assay precision.

5. Complete removal of residual liquid from the tubes after each spin is essential for proper assay performance. Continuously tap the tubes until free of all visible liquid immediately following the 2 - 3 minutes incubation with the tubes inverted.

6. Cygnus Technologies has determined that various brands of paper towels have different levels of lint and dust. Even towels and wipes that claim to be lint-free can affect results. To keep lint and dust down, we suggest gently misting the towels with either TE (10mM Tris, 1 mM EDTA) or distilled water with a standard spray bottle prior to tapping out the tubes or plate.

Limitations

Two DNA Extraction protocols are available. The DNA Extraction procedure in this kit is performed in 2mL microfuge tubes. If you have the ability to spin deep well plates at 3,200 x g and would prefer to perform the extraction in a plate, please order Catalog #D415W, *E. coli* Host Cell DNA Extraction and Amplification Kit in a 96 Well Plate.

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 DNA. The concentration of host cell DNA can be mathematically transformed for reporting residual DNA in ng/mL, ng/mg of drug product or in ng/dose.

DNA Extraction Protocol

1. Proteinase K must be diluted fresh for each assay run. Prepare only the amount of 1:10 diluted Proteinase K required for that run. For example, if the assay requires 25 tubes, add 75µL of Proteinase K to 675µL of DNA TE Buffer, (Cat # D001) or other qualified TE buffer.
2. Dilute all test samples to DNA concentrations within the analytical range of the curve and to < 20mg/mL total protein using DNA Sample Diluent (Cat# D006). All samples should be diluted at least 1:2.
3. Prepare 450µL of each Standard, test sample, and control in 2mL microfuge tube.
4. Add 25µL of diluted Proteinase K to each tube. Mix by gently vortexing for 5 seconds.
5. Incubate the standards, samples, and controls at 60°C for 30 minutes, in a dry heat block.
6. Centrifuge the tubes for 1 minute at 10,000 rpm to recover any condensation on the caps.
7. Add 450µL of Extraction Buffer to Standards, controls and samples. Vortex each tube for 5 seconds.
8. Add 1mL of Precipitation Buffer to each tube. Vortex for 5 seconds. Incubate on the bench top for 5 minutes.
9. Centrifuge the tubes at 10,000 rpm for 10 minutes.
10. Decant supernatant and invert tubes for 2-3 minutes on a lint-free wipe. Tap tubes on lightly misted lint-free wipes until free of all visible liquid.
11. Add 1.5mL of DNA Wash Buffer to each tube. Vortex for 5 seconds (the pellet may stay attached to the side of the tube). Incubate on bench top for 20 minutes. The tubes should be mixed 1-2 times during this incubation.
12. Centrifuge at 10,000 rpm for 5 minutes.
13. Decant supernatant and invert tubes for 2-3 minutes on a lint-free wipe. Tap tubes on lightly misted lint free wipes until free of visible liquid.
14. Re-suspend the pellets in 250µL of DNA TE Buffer (Cat# D001) pre-warmed to ~ 50°C.
15. Vortex each tube for 5 seconds. Incubate on the bench top for 5 minutes. An additional mixing during the incubation can aid in re-suspension.
16. The *E. coli* DNA is now ready for amplification or downstream applications requiring high quality purified DNA.

Amplification Protocol

1. The amplification reagent is prepared by combining the *E. coli* Primer Mix, Cat# D417, with PCR master mix. Cygnus recommends using *Power* SYBR® Green PCR Master Mix supplied by Life Technologies Corporation or a master mix from another licensed vendor.
2. Prepare 15µL of the amplification reagent for each well plus an additional 25% excess. For example, 96 wells + 25% = enough amplification reagent for 120 wells.

Reagent	µL/Reaction	Total volume to prepare for 120 wells (µL)
E. coli Primer Mix	2.5µL	300µL
PCR master mix	12.5µL	1500µL

3. Transfer 15µL of amplification reagent to each well of the qPCR assay plate (Cat# D004).
4. Transfer 10µL of each standard, test sample and control to the qPCR assay plate. We recommend testing each tube 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:
   1st cycle: 95°C for 10 minutes.
   35-40 cycles: 95°C for 15 seconds then 55°C for 1 minute.

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 primer set provided in this kit will amplify a single region of the *E. coli* DNA producing an amplicon of 120 base pairs with a $T_m$of 80.6 +/- 0.3.

Accuracy and Precision

E. coli DNA samples were prepared in a 10mg/mL human IgG sample matrix containing DNA Sample Diluent (Cat# D006) at various concentrations spanning the Standard Curve. Three preparations were made for each sample 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
50 pg/10µL	94%	5.0%	5.6%
5 pg/10µL	91%	5.1%	5.0%
0.5 pg/10µL	90%	10.7%	11.6%
0.05 pg/10µL	100%	13.1%	13.0%

Ordering Information/ Customer Service

To place an order or to obtain additional product information contact *Cygnus Technologies*:

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