CYGNUS TECHNOLOGIES
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E. coli Host Cell DNA Kit in 96 Well Plate

DNA Dye Binding Assay for the Measurement of Residual *E. coli* Host Cell DNA
Catalog # D410W

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. 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 and in-process monitoring. PicoGreen® based assays have been employed by many biopharmaceutical manufacturers over the years. However, in many cases proteins and buffer components can interfere in PicoGreen® dye binding to DNA 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 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

The residual *E. coli* host cell DNA assay is a DNA dye binding assay utilizing PicoGreen® dye. The procedure uses a proprietary method to isolate the residual DNA from the product and other contaminants that may interfere with the dye binding. Upon completion of the extraction of residual DNA, the samples and standards are reacted with PicoGreen® Solution. PicoGreen® dye is a DNA interculator that binds strongly to double stranded DNA. Upon binding to DNA, PicoGreen® dye fluoresces with an excitation of 485 nm and emission of 525 nm. The intensity of the fluorescent signal is proportional to the quantity of DNA in the standard or sample.

Reagents & Materials Provided

Components	Product #
DNA Extraction:	
Proteinase K, 1 x 150 µL	**D101**
DNA Sample Diluent, 1 x 30mL	**D006**
DNA Extraction Buffer, 1 x 30mL	**D105**
DNA Precipitation Buffer, 1 x 55mL	**D106**
DNA Wash Buffer, 1 x 170mL	**D104**
Deep Well Extraction Plate with Sealing Mat, 1 x 96 well plate	**D102**
PicoGreen® Assay:	
***E. coli* DNA Concentrate (10 µg/mL),** 120 µL	**D413X**
Quant-iT™ PicoGreen® dsDNA reagent Solution, 1 x 1.2mL	**D003**
Assay Plate with Plate Sealing Foil, 1 x 96 wells	**D002**

Storage & Stability

- All reagents should be stored at 2°C to 8°C for stability until the expiration date printed on the kit label.
- The Quant-iT™ PicoGreen® dsDNA reagent Solution, #D003 is light sensitive and should be stored in the kit box until use.

Materials & Equipment Required But Not Provided

- Microtiter plate reader capable of reading fluorescence at 485 & 525nm.
- Pipettors - 5µL - 1200µL.
- Benchtop centrifuge capable of spinning a microplate at 3,200 x g.
- Microtiter plate shaker with 1000rpm capability. (Recommended: THERMO Titer Plate Shaker, Model: 4625)
- Cygnus TE Buffer, Cat# D001 (Tris/EDTA used for final pellet reconstitution)
- Dry heat block with microplate adapter
- Plate sealer/pasta roller
- Vortex
- Absorbent wipes

Precautions

- For Research, Development or Manufacturing use only.
- The PicoGreen® Solution is a DNA intercalating dye and should be handled with care. Protective clothing and equipment should be worn for the duration of this procedure.
- 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 dilutions of the DNA concentrate according to the table below. Remove 500µL from tube 6 and ensure that the final volume in each tube is 1000 µL.

Tube #	*E. coli* DNA	Sample Diluent	Final Concentration
N/A	Stock	N/A	10µg/1mL
1	30µL of Stock	1470µL	200ng/mL
2	500 µL of Tube 1	1000µL	67ng/mL
3	500 µL of Tube 2	1000µL	22ng/mL
4	500 µL of Tube 3	1000µL	7.4ng/mL
5	500 µL of Tube 4	1000µL	2.5ng/mL
6	500 µL of Tube 5	1000µL	0.82ng/mL
7	0µL	1000µL	0ng/mL

Procedural Notes

1. Protein in the sample is a known interference factor in DNA extraction methods. Use of the proprietary Cygnus Sample Diluent (Cat# D006) will generally allow for acceptable DNA recovery in up to 20mg/mL of protein. If DNA detection limits allow samples to be diluted to 1mg/mL or less of total protein, Cygnus TE Buffer, Cat# D001 or a standard TE buffer may be used for dilution.
2. The PicoGreen® Solution will bind to all double stranded DNA. 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. Avoid leaning over or passing over the extraction plate as much as possible. Organize solutions and tips in a manner that minimizes the need to pass over the plate.
4. 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.
5. The Standards used in the Standard Curve **must** be extracted as well as the samples. However, the standards should **not be Proteinase K digested or subjected to the heat treatment**. Start the extraction of the standards at the addition of the Extraction Buffer.
6. Always make sure the centrifuge is balanced to within 0.5g to ensure proper assay performance. An improperly balanced centrifuge can result in loose pellets, which can adversely affect recovery and assay precision.
7. We recommend using a pasta roller to ensure a secure fit of the Sealing Mat onto the 96 deep well plate.
8. While it is possible to seal off wells for use of a partial plate, Cygnus recommends using a new plate for each DNA extraction to prevent contamination. However, if only part of the extraction plate is to be used, you can seal the unused wells with the sealing mat, and cut the mat at that point. The unused wells should remain sealed throughout the procedure. Please visit our website or contact Customer Service to purchase additional Deep Well Extraction plates, Catalog # D102.

Limitations

Two DNA Extraction kits and protocols are available. The DNA Extraction in this kit is performed in a 96 deep well plate. If you do not have the ability to spin deep well plates at 3,200 x g or would prefer to perform the extraction in microtubes please order Cygnus Catalog #D410T, *E. coli* Host Cell DNA Kit in Tubes.

Reagent Preparation Prior to Assay

1. **Preparation of Proteinase K reagent:**
   - 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 50 wells, add 75µL of Proteinase K to 675µL of TE Buffer, Cat# D001 or other qualified TE buffer.

2. **Preparation of PicoGreen® Solution:**
   - PicoGreen® Solution must be diluted fresh before each assay run.
   - Prepare only the amount of 1:25 diluted PicoGreen® required for that run. For example, if the assay requires 50 wells, add 600µL of PicoGreen® to 14.4 mL of prewarmed to 37°C, TE Buffer Cat# D001 or other qualified TE buffer.
   - Mix by gently vortexing for 5 seconds.

Assay Protocol

1. **Dilute all test samples to DNA concentrations within the analytical range of the assay and to ≤ 20mg/mL total protein using Sample Diluent (Cat# D006). All samples should be diluted at least 1:2.**
2. **Transfer 250µL of each test sample to the deep well plate (columns 4 thru 12) and perform all required spiking and diluting. If samples are being diluted in the plate, ensure final volume in wells is 250µL.**
3. **Add 12.5µL of diluted Proteinase K to each test sample well. Mix by pipetting up and down or by sealing the plate and shaking for 30 seconds on the plate shaker.**
4. **Seal the plate with the sealing mat, and incubate at 60°C for 30 minutes, in a dry heat block with a microplate adapter.**
5. **Centrifuge at 3,200 x g for 1 minute to recover any condensation on the sealing mat.**
6. **Prepare 1000µL of each Standard in 2 mL microfuge tubes during the sample incubation. Add 250µL of Standards 1-7 to columns 1-3. (Standards must not be Proteinase treated and should only be added to the plate after the Step #4 heat treatment)**
7. **Add 250µL of Extraction Buffer to Standards, controls and test samples. Reseal the plate and incubate on the plate shaker (~800rpm) for 5 minutes.**
8. **Add 500µL of Precipitation Buffer to each well. Reseal the plate and incubate on the plate shaker (~1,000rpm) for 15 minutes.**
9. **Remove the plate from the shaker and centrifuge the plate at 3,200 x g in a bench top centrifuge for 20 minutes.**
10. **Decant supernatant. Remove additional liquid by tapping the plate upside down on lint-free wipes until free of visible liquid.**
11. **Add 0.8mL of DNA Wash Buffer to the wells in the plate. Reseal the plate and incubate on the plate shaker (~1000rpm) for 10 minutes.**
12. **Centrifuge at 3,200 x g for 5 minutes.**
13. **Decant supernatant. Remove additional liquid by tapping the plate upside down on lint-free wipes until free of all visible liquid.**
14. **Add 0.8mL of DNA Wash Buffer to the wells in the plate. Reseal the plate and incubate on the plate shaker (~1000rpm) for 5 minutes.**
15. **Centrifuge at 3,200 x g for 5 minutes.**
16. **Decant supernatant. Remove additional liquid by tapping the plate upside down on lint-free wipes until free of all visible liquid.**
17. **Re-suspend the pellets by adding 250µL/well of diluted PicoGreen® Solution that has been pre-warmed to ~ 37°C.**
18. **Seal the plate and incubate at room temperature on the plate shaker (~800rpm) for 5 minutes.**
19. **Remove 240µL from each well and pipet into the Assay Plate, Cat#D002. Seal the plate with the foil provided and incubate for 5 minutes on the benchtop at room temperature.**
20. **Read the plate at Ex: 485nm, Em: 525nm, Cutoff: 515nm.**

Quality Control

- Precision on triplicate samples should yield average % coefficients of variation of less than 15% for samples in the range of 500pg/mL to 150ng/mL. CVs for samples above or below this range may be greater than 15%.
- It is recommended that each laboratory assay appropriate quality control samples in each run to ensure that all reagents and procedures are correct.

Calculation of Results

The D413X *E. coli* DNA concentrate may be used to construct a standard curve with values reported in ng/mL host cell DNA. This data reduction may be performed through computer methods using curve-fitting routines such as point-to-point, cubic spline, and 4 or 5 parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies!**

Performance Characteristics

Cygnus Technologies has validated 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 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 qualification protocols can be obtained by contacting Technical Services Department or at our web site.

Precision

DNA samples were prepared in a human IgG matrix at various concentrations spanning the Standard Curve. Four preparations were made for each sample and 32 individual extractions were collected for each point.

Concentration	% Nominal	Intra-assay CV	Inter-assay CV
100ng/mL	94%	6.7%	6.7%
25ng/mL	93%	5.2%	5.3%
5ng/mL	96%	7.5%	7.5%
1ng/mL	103%	10.9%	10.8%

Full Plate Variability Studies

Testing was performed to assess the performance of the extraction and the dye binding assay on an entire plate to judge variability. A 10ng/mL *E. coli* Host cell DNA sample was prepared in a human IgG sample matrix and loaded in all wells of the plate except for wells occupied by the Standard Curve. The plate was extracted according to the standard protocol and measured in the dye binding assay. Sample recovery averaged 99% across the entire plate with a CV of 3.7%.

Ordering Information/ Customer Service

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

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