# NS/0 Host Cell DNA Kit in Tubes

## DNA Dye Binding Assay for the Measurement of Residual NS/0 Host Cell DNA
## Catalog # D220T

## Intended Use

This kit is intended for use in determining the presence of host cell DNA contamination in products manufactured by recombinant expression in NS/0 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 NS/0 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 with the 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 NS/0 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 intercalator 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

| Component | Product # |
|---|---|
| **DNA Extraction:** | |
| **Proteinase K,** 1 x 150µL | **D101** |
| **DNA Sample Diluent,** 1 x 30mL | **D006*** |
| **DNA Extraction Buffer,** 1 x 30mL | **D108** |
| **DNA Precipitation Buffer,** 1 x 55mL | **D106** |
| **DNA Wash Buffer,** 1 x 100mL | **D103** |
| **2mL Sterile Microfuge Tubes,** 50 tubes | **D107*** |
| **PicoGreen® Assay:** | |
| **NS/0 DNA Standards Set, A-H,** 1mL Standards at 0, 0.4, 0.75, 2.5, 8, 30, 75, & 200ng/mL | **D223** |
| **Quant-iT™ PicoGreen® dsDNA reagent Solution,** 1 x 1.2mL | **D003** |
| **Assay Plate with Plate Sealing Foil,** 1 x 96 wells | **D002** |

*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.
- 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

- Pipettors – 5µL – 1200µL.
- Cygnus TE Buffer, Cat# D001 (Tris/EDTA used for final pellet reconstitution)
- 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.
- 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.

## 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.

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. 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. The standards should not be Proteinase K digested or subjected to the heat treatment step. Start the standards extraction at the addition of the Extraction Buffer. (See Step 6 of the Assay Protocol.)

5. 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.

**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 kits and protocols are available. The DNA Extraction 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 Cygnus Catalog #D220W, NS/0 Host Cell DNA Kit in 96 Well Plate.

## 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 insure that all reagents and procedures are correct.

## 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 25 tubes, 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 25 tubes, add 600µL of PicoGreen® to 14.4 mL of pre-warmed to 37°C, TE Buffer Cat# D001 or other qualified TE buffer.
   - Mix by gently vortexing for 5 seconds.

## Calculation of Results

The standards 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!**

## 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. **Prepare 500µL of each sample and control in 2mL microfuge tube.**
3. **Add 25µL of diluted Proteinase K to each tube.**
4. **Incubate the samples and controls at 60°C for 30 minutes in a dry heat block.**
5. **Centrifuge the tubes for 1 minute a 10,000 rpms to recover any condensation on the caps.**
6. **Transfer 500µL of the provided DNA standards to 2 mL microfuge tubes during the sample incubation.**
7. **Add 500µL 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 10,000rpm for 10 minutes.**
10. **Decant the supernatant and invert the tubes for 2-3 minutes on a lightly misted lint free wipe or paper towel. Tap the tubes on pre-misted lint free wipes or paper towels 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 the bench top for 20 minutes.**
12. **Centrifuge at 10,000rpm for 5 minutes.**
13. **Decant the supernatant and invert the tubes for 2-3 minutes on a lightly misted lint free wipe or paper towel. Tap the tubes on pre-misted lint free wipes or paper towel until free of visible liquid.**
14. **Re-suspend the pellets in 500µL of pre-diluted and pre-warmed PicoGreen® reagent.**
15. **Vortex each tube for 5 seconds and incubate on the bench top for 5 minutes.**
16. **Remove 240µL from each tube and pipet into the Assay Plate, Cat#D002, in duplicate. Seal the plate with the foil provided and incubate for 5 minutes on the bench top at room temperature.**
17. **Read the plate at Ex: 485nm, Em: 525nm, Cutoff: 515nm.**

## 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 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 duplicate wells were collected for each preparation.

| Concentration | % Nominal | Intra-assay CV | Inter-assay CV |
|---|---|---|---|
| 100ng/mL | 96% | 3.0% | 4.9% |
| 10ng/mL | 100% | 3.4% | 3.9% |
| 1ng/mL | 105% | 13.2% | 12.9% |

## Ordering Information/ Customer Service

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


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