



DNA Extraction Kit in Tubes

DNA Extraction Kit for the isolation of low levels of DNA Catalog # D100T

Intended Use

This kit is intended for use in isolating low levels of DNA from complex biologic solutions. 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 cultured 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. These requirements demand a sensitive method of DNA detection for compliance. However, all of the current methods for measuring low levels of residual DNA such as DNA dye binding, quantitative PCR and the Threshold® System are all severely inhibited by the presence of protein and other components commonly found in in-process and drug substance samples.

This kit is a proprietary DNA extraction procedure designed to isolate sub-picogram per milliliter levels of residual DNA from complex biological solutions in an environment free from contaminating proteins, salts and detergents allowing for timely and scientifically sound process decisions.

Reagents & Materials Provided

| Components | Product # |
|---------------------------------------|-----------|
| 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 |
| 2mL Sterile Microfuge Tubes, 50 tubes | D107* |

*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

Pipettors - 5µL - 1000µL.
Sample Diluent Buffer: Cygnus Cat # D001, or Tris/EDTA (TE Buffer).
Benchtop microfuge capable of spinning 2mL tubes at 10,000 rpm.
Dry heat block for use with tubes.
Vortex
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.

Procedural Notes

1. Cygnus suggests pre-diluting samples to ~1mg/mL of total protein, however, higher concentrations can be performed if assay qualification allows.
2. 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 DNA extraction.
3. If you also need to extract standards/calibrators for an end detection method, using PicoGreen® dye, qPCR or Threshold® System etc, and the standards are **not** in a protein matrix, these standards should not be Proteinase K digested or subjected to the heat treatment steps. Start the standards extraction at the addition of the Extraction Buffer. (See Step 5 of the Extraction Protocol.)
4. Make sure the centrifuge is balanced to ensure proper performance. An improperly balanced centrifuge can result in loose pellets, which can adversely affect recovery and assay precision.

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 #D100W, DNA Extraction Kit in a 96 Well Plate.

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 75 μ L of Proteinase K to 675 μ L of Diluent Buffer, Cat# D001 or other qualified TE buffer.

Quant-iT™ PicoGreen® dsDNA reagent trademarks of Life Technologies Corporation.

Threshold® System is a trademark of Molecular Devices, Inc.

Extraction Protocol

1. Dilute all test samples to 1- 3mg/mL total protein in Diluent Buffer, Cat. #D001 or other qualified TE Buffer.
2. Prepare 500 μ L of each sample and control in 2mL microfuge tube.
3. Add 25 μ L of diluted Proteinase K to each sample. Mix by gently vortexing for 5 seconds.
4. Incubate the samples and controls at 37°C-40°C for 20 minutes in a dry heat block.
5. Transfer 500 μ L of your DNA standards to 2mL microfuge tube during the sample incubation.
6. Add 500 μ L of Extraction Buffer to standards, controls and samples. Vortex each tube for 5 seconds.
7. Add 1mL of Precipitation Buffer to each tube. Vortex for 5 seconds. Incubate on the benchtop for 5 minutes.
8. Centrifuge the tubes at 10,000 rpm for 10 minutes.
9. Decant supernatant and invert tubes for 2-3minutes on a lint free wipe. Tap tubes on a lint-free wipe until free of visible liquid.
10. Add 1.5mL of DNA Wash Buffer to each tube. Vortex until pellet dislodges from the tube. Incubate on benchtop for 20 minutes.
11. Centrifuge at 10,000 rpm for 5 minutes.
12. Decant supernatant and invert tubes for 2-3minutes on a lint free wipe. Tap tubes on a lint-free wipe until free of visible liquid.
13. Re-suspend the pellets in Diluent/TE buffer or the diluent required for your detection method. For qPCR applications we recommend re-suspending the pellets in 250 μ L Diluent/TE buffer heated to ~50°C.
14. Vortex each tube for 5 seconds. Incubate on the benchtop for 5 minutes.
15. The DNA is now ready for downstream applications such as qPCR, dye binding assays, or any application requiring high quality purified DNA.

Ordering Information /Customer Service

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

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