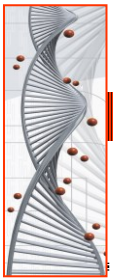

DNA Extraction Kit in Tubes

DNA Extraction Kit for the Isolation of Residual 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

Component	Product #
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***

*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 - 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.
- 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. 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. 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. 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. 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 6 of the Extraction Protocol.)

5. 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.

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

Extraction Protocol

1. **Dilute all test samples to DNA concentrations within the analytical range of the detection method 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 sample. Mix by gently vortexing for 5 seconds.**
4. **Incubate the samples and controls at 60°C for 30 minutes in a dry heat block.**
5. **Centrifuge the tubes for 1 minute at 10,000 rpms to recover and condensation on the caps.**
6. **Transfer 500µL of your DNA standards to 2mL microfuge tube 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 10,000 rpm for 10 minutes.**
10. **Decant supernatant and invert tubes for 2-3 minutes on a lightly misted lint free wipe. Tap tubes on lint-free wipes until free of 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.**
12. **Centrifuge at 10,000 rpm for 5 minutes.**
13. **Decant supernatant and invert tubes for 2-3 minutes on a lightly misted lint free wipe. Tap tubes on lint-free wipes until free of visible liquid.**
14. **Re-suspend the pellets in the desired TE buffer. For qPCR applications, we recommend re-suspending the pellets in 250µL TE buffer heated to ~50°C.**
15. **Vortex each tube for 5 seconds. Incubate on the bench top for 5 minutes.**
16. **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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