DNA Extraction Kit in 96 Well Plate

DNA Extraction Kit for the isolation of low levels of DNA
Catalog # D100W

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 binding dyes, quantitative PCR and Threshold® 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 to isolate sub-picogram per milliliter levels of residual DNA and perform the measurements 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	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*

*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.
Sample Diluent Buffer: Cygnus Cat # D001, or Tris/EDTA (TE Buffer).
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).
Dry heat block with microplate adapter.
Vortex
Plate sealer/pasta roller
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. 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. 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.)
5. 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.
6. We recommend using a pasta roller to ensure a secure fit of the Sealing Mat onto the 96 deep well plate.
7. 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 #D100T, DNA Extraction 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 Diluent Buffer, Cat# D001 or other qualified TE buffer.

Extraction Protocol

1. Dilute all test samples to 1-3mg/mL total protein in Diluent Buffer, Cat# D001 or other qualified TE Buffer.
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 37°C - 40°C for 20 minutes, in a dry heat block.
5. Add 250µL of your DNA standards (not included) to columns 1-3.
6. Add 250µL of Extraction Buffer to standards, controls and samples. Reseal the plate and incubate on the plate shaker (~780rpm) for 5 minutes.
7. Add 500µL of Precipitation Buffer to each well. Reseal the plate and incubate on the plate shaker (~1,000rpm) for 15 minutes.
8. Remove the plate from the shaker and centrifuge the plate at 3,200 x g in a bench top centrifuge for 20 minutes.
9. Decant supernatant. Remove additional liquid by tapping the plate upside down on a lint-free wipe until free of visible liquid.
10. 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.
11. Centrifuge at 3,200 x g for 5 minutes.
12. Decant supernatant. Remove additional liquid by tapping the plate upside down on a lint-free wipe until free of all visible liquid.
13. 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.
14. Centrifuge at 3,200 x g for 5 minutes.
15. Decant supernatant. Remove additional liquid by tapping the plate upside down on a lint-free wipe until free of all visible liquid.
16. Re-suspend the pellets in the desired Diluent/TE buffer. Heating this buffer may aid in dissolving the DNA pellet. For qPCR applications we recommend re-suspending the pellets in 125µL of Diluent/TE buffer heated to ~50°C.
17. Seal the plate and incubate at room temperature on the plate shaker (~780rpm) for 3 - 5 minutes.
18. 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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