EIV82024E

ExCell Bio

ResiQuant® Universal DNA Residual Sample Pretreatment Kit

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CRB00-0011S
	CRB00-0011
	CRB00-0012

PRODUCT DESCRIPTION

The Universal DNA Residual Sample Pretreatment Kit is designed to extract DNA from biopharmaceutical products at various stages of processing. This kit employs a proprietary DNA extraction procedure capable of isolating picogram per milliliter level of residual DNA from biological solutions. The process eliminates others components like proteins, salts, and detergents, enabling quick and efficient residual DNA detection.

In order to quantify residual host cell DNA, it is recommended to use this kit together with corresponding quantitative DNA kit.

PERFORMANCE, APPLICATION AND RESTRICTION

This kit is designed to isolate residual host cell DNA from variant biological solutions.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Serial Number	Components	CRB00-0011 (50T)	CRB00-0012 (100T)	CRB00-0011S (25T)
Box 1	Lysis/Binding Buffer	10 mL	2 × 10 mL	5 mL
	Wash Buffer	28 mL	2 × 28 mL	14 mL
	Elution Buffer	5 mL	10 mL	2.5 mL
	Proteinase K Buffer	5 mL	2 × 5 mL	2.5 mL
	Magnetic Bead Solution	250 μL	2 × 250 μL	125 μL
Box 2	Proteinase K	1 mL	2 × 1 mL	500 μL
	Yeast tRNA	10 μL	2 × 10 μL	5 μL
	Glycogen	450 μL	2 × 450 μL	225 μL

Storage: Box 1 should be stored at room temperature, and Box 2 at -40℃ to -18℃.

Validity: 12 months under specified storage conditions.

Transportation: Box 1 is transported at room temperature, and Box 2 is transported with dry ice .

EXPERIMENTAL PREPARATION

Materials & Equipment required but not included

- Vortex shaker;
- Magnetic rack;
- Centrifuge;
- Water bath or Dry bath;
- 1.5 mL tubes;
- Ethanol (analytical);
- 1×PBS (pH 7.4, sterile filtered);
- 5M NaCl (excellent grade, sterile filtered);
- Ultrapure water (PCR grade).

Reagent Preparation Prior to Assay

- Lysis/Binding Buffer is provided as a concentrate. If precipitation occurs, incubate the solution at 37°C for 30 minutes to dissolve the precipitate;
- Add 100% ethanol to the Wash Buffer as indicated on the label, and mix the solution togethergently;
- 70% ethanol should be freshly prepared before each extraction;

EXPERIMENTAL PROCEDURE

Sample preparation

- Sample dilution (if necessary): Samples from the upstream process usually contain high level of residual DNA. The suitability study is suggested to obtain an optimal condition for an accurate test. The samples can be diluted using 1×PBS.
- Negative sample (NEG): NEG is set up in each experiment to monitor the whole process as a theoretical negative;
- Extraction/recovery Control (ERC): ERC is used to evaluate the assay efficiency and can be prepared by spiking the test sample with proper amount of standard DNA.

Lysis/Binding

1. Add 100 µL of each sample to a 1.5mL tube;
2. Add 20 µL Proteinase K, 100 µL Proteinase K Buffer and 20 µL 5M NaCl to each tube, vortex the tubes for 5-10 seconds to mix the solution thoroughly, and then incubate the tubes at 70℃ for 15 minutes.
3. Prepare the Lysis/Binding solution (for one sample):

200 µL Lysis/Binding Buffer + 9 µL Glycogen + 0.2 µL Yeast tRNA, vortex the tubes for 5-10 seconds to mix the solution thoroughly, and add 209.2 µL to each tube;

4. Add 200 µL 100% isopropanol (IPA) to each tube;
5. Vortex Magnetic Bead Solution for 30 seconds, add 5 µL beads to each tube, and vortex or shake the tube vigorously for 10 minutes at room temperature.

Note: keep the Magnetic Beads Solution consistent in the process of adding the beads to the test samples.

6. Place the tubes on a magnetic rack for 2-5 minutes to let the beads assembled to form a tight pellet and clear the liquid;
7. Carefully remove the liquid without touching the beads;

Wash

1. Add 700 µL of Wash Buffer (check if ethanol has been added) to each tube, vortex the tubes for 30 seconds to re-suspend the beads;
2. After assemble the beads to the bottom by short-time spin, place the tubes on the magnetic rack for 2-5 minutes to clear the solution;
3. Carefully remove the liquid without touching the beads.
4. Add 700 µL of 70% ethanol to each tube, vortex for 30 seconds or invert for 10 times to re-suspend the magnetic beads;
5. After assemble the beads to the bottom by short-time spin, place the tubes on the magnetic rack for 2-5 minutes to clear the solution;
6. Remove the liquid from the tube without touching the beads;
7. Continue to keep the tubes on the magnetic rack for 1 min and remove the remaining liquid;
8. Keep the tubes on the magnetic stand with the lid open for 5-10 minutes (keep an eye on the magnetic beads to make sure it doesn't crack).

Note: Over-drying of magnetic beads may affect the recovery rate of the DNA.

Elution

1. Move the tubes to a regular rack and add 100 µL of Elution Buffer to the beads;
2. Vortex the tubes to re-suspend the beads and incubated at 70℃ for 15 minutes, vortex the tubes every 5 minutes during the incubation;
3. After the incubation, let the tubes cool down to room temperature, spin the tubes to assemble the beads to the bottom;
4. Keep the tubes on the magnetic rack for 2-5 minutes to separate the beads from the solution;
5. Carefully transfer the liquid without touching the beads to a new tube as read-to-use test sample;
6. Save the test sample at -80℃ to -20℃ in case that the assay is not performed right away.

Analyses of test results

The recovery rate is usually be between 70% to 130%.

Operation details

- When separating the magnetic beads on the magnetic stand, the tubes can be rotated left and right to make the adsorption of the magnetic beads more concentrated;
- During the wash or elution of the magnetic beads, spin briefly in a mini centrifuge after each vortex to ensure that no magnetic beads are attached to the cap and wall of the tubes;
- Test the purified DNA immediately to ensure the accuracy of the results.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.

2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.