EIV92025E

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 assay kit.

PERFORMANCE, APPLICATION AND RESTRICTION

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

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

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

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

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

1. Vortex shaker.
2. Magnetic rack.
3. Centrifuge.
4. Water bath or Dry bath.
5. 1.5 mL tubes.
6. Ethanol (analytical).
7. Isopropanol (analytical).
8. 1× PBS (pH 7.4, sterile filtered).
9. 5M NaCl (excellent grade, sterile filtered).
10. Ultrapure water (PCR grade).

Reagent Preparation Prior to Assay

1. Lysis/Binding Buffer is provided as a concentrate. If precipitation occurs, incubate the solution at 37°C for 10 minutes to dissolve the precipitate.
2. Add 100% ethanol to the Wash Buffer as indicated on the label, and mix the solution together gently.
3. 70% ethanol should be freshly prepared before each extraction.
4. Prepare the lysis/binding solution freshly for each individual sample prior to extraction as follows:
   200 µL Lysis/Binding Buffer + 9 µL Glycogen + 0.2 µL Yeast tRNA (Yeast tRNA is not recommended for yeast residual DNA extraction), vortex thoroughly, briefly centrifuge, and collect the solution at the bottom of the tube.

EXPERIMENTAL PROCEDURE

Sample preparation

1. 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.
2. Negative sample (NEG): NEG is set up in each experiment to monitor the whole process as a theoretical negative control.

3. 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, The DNA spiking amount for the specific samples can be designed based on the previous studies.

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, mix thoroughly by inverting or vortexing.

***Note**: For large sample volumes, Proteinase K Buffer and 5 M NaCl may be premixed in proportion, thoroughly mixed, then incubated at 70 ° C for 1 minute to dissolve precipitates.*

3. Incubate at 70°C for 15 minutes, followed by 5 minutes at room temperature, and then briefly centrifuge.
4. Add freshly prepared lysis/binding buffer to the aforementioned tubes (200 µL Lysis/Binding Buffer + 9 µL Glycogen + 0.2 µL Yeast tRNA, Yeast tRNA is not recommended for yeast residual DNA extraction), vortex thoroughly and briefly centrifuge.
5. Add 200 µL 100% isopropanol (IPA) to each tube, vortex thoroughly and briefly centrifuge.
6. 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.*

7. Place the tubes on a magnetic rack for 1 minute to let the beads assembled to form a tight pellet and clear the liquid.
8. Carefully remove the liquid without touching the beads.

Wash Buffer

1. Add 700 µL of the Wash Buffer (check if ethanol has been added) to each tube, vortex the tubes for 30 seconds to resuspend the beads.
2. After assemble the beads to the bottom by short-time spin, place the tubes on the magnetic rack for 1 minute to clear the solution.
3. Carefully remove the liquid without touching the beads.

70% Ethanol

1. Add 700 µL of the 70% ethanol to each tube, vortex for 30 seconds or invert for 10 times to resuspend the magnetic beads.
2. After assemble the beads to the bottom by short-time spin，place the tubes on the magnetic rack for 1 minute

to clear the solution.

3. Remove the liquid from the tube without touching the beads.
4. Continue to keep the tubes on the magnetic rack for 1 minute and remove the remaining liquid.
5. Keep the tubes on the magnetic rack with the lid open for 2 to 3 minutes (keep an eye on the magnetic beads to avoid over-drying the beads).

Note: *Over-drying the magnetic beads may decrease the recovery rate.*

Elution

1. Move the tubes to a regular rack and add 100 µL of the Elution Buffer to the beads.
2. Vortex the tubes to resuspend the beads and incubated at 70℃ for 15 minutes, vortex the tubes every 3 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 minutes to effectively separate the beads from the solution. Transfer the solution to a fresh 1.5 mL microcentrifuge tube, accurately record the transferred volume, and proceed promptly with sample analysis.

Analyses of test results

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

Operation details

1. When separating the magnetic beads on the magnetic rack, the tubes can be rotated left and right to make the adsorption of the magnetic beads more concentrated.
2. 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.
3. It is recommended to test the purified DNA immediately.

FAQ

Question	Reason	Solution
Low recovery rate of sample purification	Over-dried beads after washing impairing DNA elution	Limit drying time to a half to 1 minute under high temperature/dry conditions, or 1 to 3 minutes under low temperature/humid conditions
	Beads adhering to the tube wall during elution, which hinders proper mixing	Vortex tubes after adding elution buffer. If beads remain attached, incubate at 70°C for 2minutes, and then vortex again
	Magnetic beads are lost during the washing process	In case that the beads gathering at tube bottom, gently pipette to resuspend the beads and let the beads able to be captured by the magnetic rack
	High protein content in sample	Dilute the sample and increase the dosage/incubation time of the Proteinase K
	Low sample pH	Adjust pH to neutral range
	Low salt concentration	Adjust using 5M NaCl
Inconsistent recovery results	Residual beads in the eluate	Repeat centrifugation and collect the supernatant
	Inaccurate elution volume	Regularly calibrate pipettes; use low-retention filtered tips

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.