SunLong Biotech Co.,LTD
Tel: 0086-571- 56623320 Fax:0086-571- 56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Magnetic Residual DNA Sample Preparation Kit (100T)

Cat No.:CG-HCD100T

1. Application

This kit is suitable for the preparatory treatment of residual DNA detection in biological product samples. Through chemical lysis and adsorption of magnetic beads, it can extract ultra-low amount of DNA from various sample types to the maximum extent.

This kit is recommended for sample preparation for host cell DNA residual（qPCR-based）detection kits，including:

CHO Host Cell Dna Residue Detection kit

HEK293 Host Cell Dna Residue Detection kit

NS0 Host Cell Dna Residue Detection kit

Vero Host Cell Dna Residue Detection kit

E.Coli Host Cell Dna Residue Detection kit

Pichia Pastoris Host Cell Dna Residue Detection kit

2. Product Components and Storage

After receiving the kit, please check the contents according to the table provided. If the quantity is correct, storage them under the corresponding conditions.

This kit provides reagents sufficient for analysis of 100 samples.

	Components	Quantity	Storage
1	Lysis Buffer	10mL/Vial*1	Room Temperature
2	Wash Solution A	25mL/Vial*1 Add 25mL Anhydrous Ethanol in the first use	
3	Wash Solution B	15mL/Vial*1 Add 45mL Anhydrous Ethanol in the first use	
4	Elution Buffer	10mL/Vial*1	
5	Magnetic Bead Suspension	1mL/Tube*2	4℃

6	Proteinase K[1]	1mL/Tube*2	-20℃
7	Glycogen	0.8mL/Tube*1	
8	POLY(A) Potassium Salt	0.5mL/Tube*1	

Note: [1]To prevent any potential impact on its effect caused by repeated freeze-thaw cycles, it is recommended to aliquot the component upon first use.

3. Equipment and Consumables Required But Not Provide

1) Magnetic Separation Rack
2) Vortex mixer
3) Mini centrifuge or standard centrifuge compatible with 2mL centrifuge tubes
4) Water bath
5) 10μL-1000μL pipette tips with low retention filter (RNase/DNase Free, sterile) and corresponding pipettor
6) 2mL low retention Centrifuge tubes(RNase-/DNase-Free, sterile)
7) Anhydrous Ethanol (Analytical Pure)
8) Isopropanol
9) 1×PBS Buffer (pH 7.4, free of Mg2+ and Ca2+)
10) RNase-Free H_2O

4. Preparation before Assay

1) Please read the manual thoroughly before use and follow the instruction strictly.
2) For your safety and health, please wear lab coat and disposable gloves when conducting the assay.
3) This assay is recommended to be conducted in a biosafety cabinet or clean bench.
4) To avoid cross-contamination,use disposable sterilized tips during the experiment.
5) During experimentation, carefully open and close sample tubes. Do not open all simultaneously to prevent splash-out and cross-contamination.
6) Prior to each experiment, please check all solutions stored at room temperature to see if there is any precipitate, especially in winter. If any precipitation is found in the bottle, it can be dissolved by shaking it in a 37℃ water bath.
7) After adding anhydrous ethanol to Wash Solution A and Wash Solution B, please make a mark on the bottle. After each experiment, please tighten the cap and seal it with sealing film to prevent ethanol evaporation.

5. Extraction Protocol

1) To prepare for the first use, add 25mL Anhydrous Ethanol to Washing Solution A, then add 45mL Anhydrous Ethanol to Washing Solution B. Mix well and mark the bottle respectively.
2) Prior to assay, set the water bath at 56℃. (If there is only one water bath, set it to 56℃. If there are two water baths, set one at 56℃ and the other at 65℃, and preheat the Elution Buffer in the 65℃ water bath.)
3) Sample Preparation

   3.1 For test samples from the early purification process or samples with high DNA contents, it is recommended to extract the samples after appropriate dilution.

   3.2 If the sample is a dry powder, it is recommended to dilute it with enzyme-free water or 1×PBS buffer solution (pH 7.4, without Mg2+ and Ca2+) to obtain a concentration of 10mg/mL for extraction.

   3.3 For sample solutions with a complicated background, it is recommended to conduct spike recovery to eliminate potential matrix interference.
4) For each sample, we recommend setting a Negative Control Sample (NCS), an Extraction Recovery Control (ERC) for cross-contamination checkduring sample processing and DNA extraction accuracy verification.

   Note: During ERC test, a certain amount of standard is added to each sample to make the total DNA quantity 2-10 times the DNA quantity measured in the test sample.
5) Add 100μL sample into 2ml RNase-/DNase-free and sterile Centrifuge Tubes respectively, and it is recommended to perform this experiment in triplicate for each sample. Add 10μL of Proteinase K and 100μL Lysis Buffer, vortex to mix well, and then centrifuge for 5 seconds.

   Note: For the concentrations of sample between 0-100mg/mL, we recommend adding 10μL proteinase K; For the concentrations of sample between 100-200mg/mL, we recommend adding 20μL proteinase K.
6) Incubate sample at 56℃ for 30minutes.

   Note: If there is only one water bath, set the temperature of the water bath to 65°C after this step is completed, and then preheat the elution buffer in the water bath.
7) After the incubation, allow the tubes to return to room temperature. Then add 8 μ L Glycogen, 5 μ LPOLY(A) Potassium Salt, and 400 μ L Isopropanol to each tube. Vortex to mix well, then centrifuge for 5 seconds.
8) Spiral the magnetic beads, ensuring all beads are suspended. Add 20 μ L of beads into the first tube, then immediately vortex to mix. Repeat this process for the remaining tubes.

Note: When adding magnetic beads, make sure all beads are suspended. It is recommended to vortex sample every 4 additions.

9) Let the tubes stand for 10 minutes and vortex for 10 seconds every 3minutes.
10) Centrifuge the solution for 5 seconds at 4000rpm with Mini or Standard Centrifuge, place it on a magnetic rack for 1-2 minutes until the solution is clarified. Remove the supernatant carefully with a pipettor.
11) After adding 400μL of Wash Solution A into each tube, mix well by Vortex and ensure that all magnetic beads are suspended. Centrifuge 5 seconds, and place it on a magnetic rack for 1-2 minutes until the solution is clarified. Remove the supernatant using a pipettor with caution.
    Note: For the first use, make sure 25mL Anhydrous Ethanol has already been added into Wash Solution A.
12) Adding 500μL of Wash Solution B into each tube, mix well with Vortex and ensure that all magnetic beads are suspended. Centrifuge 5 seconds, and place it on a magnetic rack for 1-2 minutes until the solution is clarified. Remove the supernatant using a pipettor carefully and make sure there is no residue in the tube.
    Note:For the first use, make sure 45mL Anhydrous Ethanol has already been added into Wash Solution B.
13) Open the lid，and place it at room temperature for 3-5minutes and monitor the surface of magnetic beads. Once the surface of beads lost its reflective appearance，add 100μL of preheated Elution Buffer immediately and Vortex the tube to suspend all beads.
14) **Note**:To ensure that the ethanol has fully evaporated and that the magnetic beads are not over-dried, this step requires careful observation on beads. The residual ethanol can interfere with the subsequent qPCR experiment, while over drying can reduce DNA recovery rate.
15) To suspend all magnetic beads, incubate sample at 65℃ for 6 minutes and mix well with vortex every 2 minutes.
    Note:To avoid the centrifuge tube lid from popping open during heating, when incubating samples at 65℃，it is recommended to place a weight on top of the centrifuge tube or wrap it with sealing film.
16) After incubation is completed, allow the tubes to return to room temperature.. Then centrifuge it for 5-10 seconds and place it on a magnetic rack for 1-2 minutes. Using pipettor to transfer Elution Buffer into a new low retention centrifuge tube (RNase/DNase-Free, sterile).
17) We recommend proceeding immediately with the subsequent qPCR procedure for the sample, or storing it at 4° C for up to 6 hours, or at -20° C for 24 hours.