



DNA Sample Preparation Kit (100T, Magnetic Bead Method)

Applicable for: Manual Extraction with Magnetic

Rack and Automated Nucleic Acid Extractors

Cat#: CG-DP100

1. Application

This kit is designed 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 to prepare sample for host cell DNA residual (qPCR-based) detection kits, including:

CHO Host Cell Dna Residue Detection kit (CH-D050T/CH-D100T)

HEK293 Host Cell Dna Residue Detection kit (HK-D050T/HK-D100T)

NS0 Host Cell Dna Residue Detection kit (NS-D050T/NS-D100T)

Vero Host Cell Dna Residue Detection kit (VE-D050T/VE-D100T)

E.oli Host Cell Dna Residue Detection kit (EC-D050T/EC-D100T)

Pichia Pastoris Host Cell Dna Residue Detection kit (PP-D050T/PP-D100T)

Ogataea polymorpha Host Cell Dna Residue Detection kit (HP-D050T/HP-D100T)

Saccharomyces cerevisiae Host Cell Dna Residue Detection kit (SC-D050T/SC-D100T)

Hela&Human Host Cell Dna Residue Detection kit (HL-D050T/HL-D100T)

MDCK Host Cell Dna Residue Detection kit (CK-D050T/CK-D100T)

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



2	Wash Solution A	25mL/Vial*1 Add 25mL Anhydrous Ethanol in the first use	Temperature
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	1.5mL/Tube*2	2-8°C
6	Proteinase K ^[1]	1mL/Tube*2	-20°C
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 Rack (for manual extraction) or automated nucleic acid extractor
- 2) Vortex mixer
- 3) Mini centrifuge or standard centrifuge compatible with 2mL centrifuge tubes
- 4) Water bath
- 5) Low-binding 96-well plate (for automated nucleic acid extractor); filter sterile RNase-free pipette tips (10µL–1000µL) and matching pipettors
- 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 H₂O

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°C 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. DNA Extraction (Manual Extraction with Magnetic Rack)

Pre-Experiment Preparation

- 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°C. (If there is only one water bath, set it to 56°C. If there are two water baths, set one at 56°C and the other at 65°C, and preheat the Elution Buffer in the 65°C water bath.)
- 3) Sample Preprocessing
 - a. For test samples from the early purification process or samples with high DNA contents, it is recommended to extract the samples after appropriate dilution.
 - b. 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 Mg^{2+} and Ca^{2+}) to obtain a concentration of 10mg/mL for extraction.
 - c. 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 check during 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.

DNA Extraction Procedure

- 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 is 0-100mg/mL, it is recommended to add 10 μ L proteinase K; For the concentrations of sample is 100-200mg/mL, it is recommend to add 20 μ L proteinase K.

- 6) Incubate sample at 56°C 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 L POLY(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 400 μ L 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 500 μ L 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 100 μ L of preheated Elution Buffer immediately and Vortex the tube to suspend all beads.



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.

- 14) To suspend all magnetic beads, incubate sample at 65°C 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°C, it is recommended to place a weight on top of the centrifuge tube or wrap it with sealing film.

- 15) 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).
- 16) We recommend proceeding immediately with the subsequent qPCR procedure for the sample, or storing it at 2-8°C for up to 6 hours, or at -20°C for 24 hours

6. DNA Extraction (Automated Nucleic Acid Extractor)

Pre-Experiment Preparation

- 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°C. (If there is only one water bath, set it to 56°C.)
- 3) Sample Preprocessing.
 - a. For test samples from the early purification process or samples with high DNA contents, it is recommended to extract the samples after appropriate dilution.
 - b. 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 Mg^{2+} and Ca^{2+}) to obtain a concentration of 10mg/mL for extraction.
 - c. 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 check during 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) DNA Extraction Preparation (Pre-load all reagents into the 96-well plate)

Sample Group 1							Sample Group 2					
	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1						Sample 1+ERC					
B	Sample 2						Sample 2+ERC					
C	Sample 3						Sample 3+ERC					
D	Sample 4						Sample 4+ERC					
E	Sample 5						Sample 5+ERC					
F	Sample 6						Sample 6+ERC					
G	Sample 7						Sample 7+ERC					
H	NCS											
Component Description	Glycogen	Wash Solution A	Wash Solution B	Elution Buffer	Magnetic Bead Suspension		Glycogen					
	POLY(A) Potassium Salt						POLY(A) Potassium Salt	Wash Solution A	Wash Solution B	Elution Buffer	Magnetic Bead Suspension	
	Isopropanol						Isopropanol					

Columns 1 & 7: 8μL glycogen per well, 5μL potassium poly(A), 400μL isopropanol per well

Columns 2 & 8: 400μL Wash Buffer A (supplemented with ethanol) per well

Columns 3 & 9: 500μL Wash Buffer B (supplemented with ethanol) per well

Columns 4 & 10: 100μL Elution Buffer per well

Columns 5 & 11: 30μL magnetic beads per well

6) Instrument Settings

Adjust the heating temperature of elution wells to 65 °C.

DNA Extraction Procedure

7) Add 100μL sample into 2mL RNase-/DNase-free and sterile Centrifuge Tubes. 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 is 0-100mg/mL, it is recommended to add 10μL proteinase K; For the concentrations of sample is 100-200mg/mL, it is recommended to add 20μL proteinase K.

8) Incubate sample at 56°C for 30minutes.

9) After the incubation, allow the tubes to return to room temperature. Transfer all pre-treated samples into Columns 1 and 7 of the 96-well plate, then mix thoroughly by shaking.

10) Aspirate 20μL magnetic beads from Column 5 into Column 1, and 20μL magnetic beads from Column 11 into Column 7. Shake to mix well and incubate for 10 min; shake for 5 seconds



every 3 minutes during this period.

- 11) Transfer the magnetic beads from Column 1 to Column 2, and beads from Column 7 to Column 8. Shake thoroughly to fully resuspend all beads. Insert the magnetic rod and let stand for 1-2 min until the solution becomes clear.

Note: For the first use, add 25mL Anhydrous Ethanol to Wash Solution A.

- 12) Transfer the magnetic beads from Column 2 to Column 3, and beads from Column 8 to Column 9. Shake thoroughly to fully resuspend all beads. Insert the magnetic rod and let stand for 1-2 minutes until the solution becomes clear.

Note: For the first use, add 45mL Anhydrous Ethanol to Wash Solution B.

- 13) Lift the magnetic rod with beads out of the liquid surface and incubate at room temperature for 3-5 minutes. Observe the bead surface; once the surface loses luster, immediately add pre-warmed elution buffer (preheated to 65°C) from Columns 4 and 10. Shake vigorously to fully resuspend all magnetic beads.

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.

- 14) Incubate at 65°C for 6 min. Mix thoroughly every 2 min to keep magnetic beads fully resuspended..
- 15) After incubation is completed, allow the tubes to return to room temperature. then transfer the eluate into a new RNase-free, sterile, low-binding microcentrifuge tube.
- 16) It is recommended to proceed immediately with the subsequent qPCR assay for the sample. Alternatively, sample may be stored at 2-8°C for up to 6 hours or at -20°C for up to 24 hours.