

MagicPure[®] Host Cell Residual DNA Kit

Please read the manual carefully before use.

Cat. No. EH201

Version No. Version 1.1

Storage: The kit is stable at 15°C-30°C for one year. Carrier RNA (1 µg/µl) is stable at -18°C or below for one year.

Description

This kit utilizes a unique lysis buffer to release trace DNA. The released host cell residual DNA is effectively purified after specifically binding to silica magnetic beads. For extraction of trace DNA fragments from host cells (CHO, Vero, NSO, MDCK, etc.) from ≤200 µl biological samples. The isolated DNA with high purity can be applied in PCR, RT-PCR, qPCR, qRT-PCR, etc. This kit is compatible with a high-throughput magnetic-rod nucleic acid extractor.

Features

- Simple and fast, no centrifugation required
- High yield, high purity

Kit contents

Component	EH201-01/11 (50 rxns)
Binding Buffer 42 (BB42)	15 ml
Clean Buffer 42 (CB42)	25 ml
Wash Buffer 42 (WB42)	6 ml
Nuclease free Water	15 ml
Host Cell Residual DNA Beads	2 ml
Proteinase K (20mg/ml)	1 ml
Carrier RNA (1µg/µl)	150 µl
Magnetic Stand (16 hole)	1 each /-

Sample Requirements

- Avoid repeated freezing and thawing.
- When the sample is a biological intermediate, please ensure that the pH value is neutral. Use sodium hydroxide or hydrochloric acid to adjust the pH of the sample to neutral (pH 6.0-8.0) before extraction.
- To ensure that the experimental results are credible:
It is recommended to increase the extraction recovery control (ERC) sample, and perform nucleic acid extraction and quantitative detection steps simultaneously. To determine the recovery efficiency of nucleic acid extraction.
It is recommended to add negative control samples (NCS), and simultaneously perform nucleic acid extraction and quantitative detection steps. It is used to determine the presence or absence of process contamination in nucleic acid extraction.

The suggested sample preparation method is as follows:

- (a) The extraction recovery control (ERC) sample: add 20 µl of host DNA standard to 180 µl of the sample to be tested and mix well to serve as ERC;
- (b) The negative control sample (NCS): 200 µl of 1×TE Buffer (DNA diluent or basic solvent for biological products) as NCS.



Procedures

Before use, add 5 ml isopropanol (analytical reagent) into BB42, and 25 ml and 24 ml absolute ethanol (Analytical Reagent) into CB42 and WB42, respectively.

1. Sample processing

- (a) Take a sterile 1.5 ml microcentrifuge tube. Add 300 μ l BB42, 20 μ l Proteinase K, 2 μ l Carrier RNA (1 μ g/ μ l).
- (b) Add 200 μ l of the liquid sample to be tested (the sample volume is less than 200 μ l, which can be supplemented with 1 \times PBS or 0.9% NaCl), vortex and mix for 5 seconds, and then incubate at 56°C for 15 minutes after brief-centrifugation.
- (c) Add 40 μ l of magnetic bead suspension (note: vortex to mix the magnetic beads before use), and vortex for 30 seconds. Incubate at room temperature for 10 minutes, mixing upside down 3-5 times.
2. Briefly spin the tube for 10 seconds using a mini centrifuge to collect the solution at the bottom. Place the tube on a magnetic stand and stay still for 30 seconds. Discard the supernatant without disturbing the magnetic beads.
3. Remove the microcentrifuge tube, add 800 μ l CB42 (check whether absolute ethanol has been added before use), vortex and mix for 15 seconds, and then perform the magnetic separation. Briefly centrifuge the tube for 10 seconds using a mini centrifuge. Place the tube on a magnetic stand and stay still for 30 seconds. Discard the supernatant without disturbing the magnetic beads.
4. Remove the microcentrifuge tube, add 500 μ l WB42 (check whether absolute ethanol has been added before use), vortex and mix for 15 seconds, and then perform the magnetic separation. Briefly centrifuge the tube for 10 seconds using a mini centrifuge. Place the tube on a magnetic stand and stay still for 30 seconds. Discard the supernatant without disturbing the magnetic beads.
5. Briefly spin the tube for 10 seconds using a mini centrifuge. Place the tube on a magnetic stand. Discard the residual liquid at the bottom of the tube using a small-volume pipette. Open the cap and incubate the tube on a dry bath at 65°C for 5 minutes to dry the magnetic beads. (Incomplete drying will significantly reduce elution efficiency. If a dry bath is unavailable, air-dry the tube on the magnetic stand at room temperature for 15-20 minutes.)
6. Remove the microcentrifuge tube, add 200 μ l Nuclease-free Water for elution, mix by vortexing or pipetting for 1 minute, then incubate at 65°C for 5 minutes, during which time, vortex gently 2-3 times to suspend the magnetic beads.
7. Place the microcentrifuge tube on a magnetic stand to separate the magnetic beads, pipet the liquid other than the magnetic beads into a sterile 1.5 ml microcentrifuge tube, avoid pipetting the magnetic beads, and store the DNA solution at -70°C.

Notes

- Avoid repeated thawing and freezing samples to ensure high-quality extracted nucleic acid.
- Use Nuclease-free sterile microcentrifuge tubes and pipette tips to avoid sample nucleic acid degradation.
- During the extraction process, after magnetic separation, be sure to aspirate and discard the liquid in the microcentrifuge tube and near the tube cap at the same time.
- Thoroughly dry beads and the tube before elution to avoid residual ethanol interfering with downstream applications.
- For recommendations on automated extraction, please contact Technical Supports from TransGen Biotech.

For research use only, not for clinical diagnosis.

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