60 Hickory Drive
Waltham, MA 02451
United States

Tel: (617) 665-7333
support@kactusbio.us
kactusbio.com

Host Cell Residual DNA (Magnetic Beads) Sample Preparation Kit

CATALOG #DK-SP001, 100 Preps

INTRODUCTION

The Residual Host Cell DNA (Magnetic Bead-Based) Sample Preparation Kit is designed for extracting trace DNA from diverse biological products, enabling detection of residual host nucleic acids and mycoplasma DNA. Combining chemical lysis with magnetic bead-based nucleic acid extraction technology, the kit supports both manual extraction and automated instrument-based processing.

KIT COMPONENTS

	Component	Size	Storage Conditions
Part I	Lysis Buffer	12 mL×1 bottle	2-8°C
	Wash Buffer	30 mL×1 bottle	
	Elution Buffer	12 mL×1 bottle	
	Magnetic Beads	1 mL×2 vials	
	Proteinase K (PK)	1 mL×1 vial	
	Binding Solution	12 mL×2 bottles	
	Sample Diluent	12 mL×1 bottle	
	5M NaCl	600μL×2 vials	
Part II	Glycogen	800 μL×2 vials	-20°C

STORAGE AND SHELF LIFE

Unopened reagents maintain stability for 12 months from the date of manufacture when stored as recommended.

REQUIRED MATERIALS NOT SUPPLIED

- Ethanol (AR), Isopropanol (AR), 1M NaOH, 1M HCl
- Vortex Mixer, Magnetic stand, Centrifuge, Water bath, Pipettor
- 1.5 mL sterile and low retention centrifuge tube, sterile pipette tips, disposable gloves.

WORKFLOW FOR DNA EXTRACTION

Prepare the reagents and samples

1. **Prepare the kit.** Equilibrate all components in kit to room temperature before use.
2. **Prepare the reagents below.**
   a) 80% fresh ethanol.

60 Hickory Drive
Waltham, MA 02451
United States

Tel: (617) 665-7333
support@kactusbio.us
kactusbio.com

b) Working Binding Solution. Note: Do not add Yeast tRNA in Working Binding Solution when processing yeast DNA samples.

Components	For single reaction
Binding Solution	200 µL
Glycogen	9 µL
Yeast tRNA	0.2 µL

c) Wash Buffer with ethanol (1:1). Before first use, please add 30 mL 100% ethanol to Wash Buffer bottle.

3. Set water baths to 65 °C and 70°C.
4. Sample Preparation:
   a) Sample dilution: Use Sample Diluent to dilute samples from the early purification process for which DNA may exceed the assay's detection limit. The recommended diluent ratio is 1:100 or 1:1000.
   b) For dry powder sample: Use Sample Diluent to dilute the dry powder sample into 10 mg/mL or 100 mg/mL generally.
   c) Adjust the pH to 6-8 if necessary, using 1M NaOH or 1M HCl.
   d) It is recommended that each sample undergo three parallel DNA extraction procedures.
   e) Extraction/recovery control (ERC): Spike each sample with positive control DNA at 2-10X the sample's endogenous DNA level, using ≤10% of the total sample volume.
   f) Negative extraction control: Include one blank control per extraction. Use 1X PBS buffer or DNA diluent buffer alongside test samples to monitor contamination.
5. Sample preparation for mycoplasma DNA detection
   a) Cell samples (≤ 1×10^6 cells):
      i. Collect cell pellet and supernatant for the test:
         1. Centrifuge the sample at 500×g for 5 min. Transfer the supernatant to a new tube. Keep the cell pellet.
         2. Centrifuge supernatant at 20,000×g for 10 minutes. Discard the supernatant until 20-30 µL remains.
         3. Resuspend the cell pellet and supernatant. Adjust the final volumes to 100 µL with DNA dilution buffer.
      ii. Collect cell supernatant for the test:
         1. Centrifuge the sample at 500×g for 5 minutes. Transfer the supernatant to a new tube.
         2. Centrifuge supernatant at 20,000×g for 10minutes. Discard the supernatant until 100 µL remains. Resuspend and then it is ready for testing.
   b) Non-cellular sample (e.g. cell culture medium).
      i. Centrifuge the sample at 20 000×g for 10minutes. Discard the supernatant until 100 µL remains. Resuspend and then it is ready for testing.

60 Hickory Drive
Waltham, MA 02451
United States

Tel: (617) 665-7333
support@kactusbio.us
kactusbio.com

Protocol for Manual DNA extraction

1. Digest the samples and controls.
    a) Add 100 µL sample to a 1.5 mL tube.
    b) Add 10 µL 5M NaCl, 100 µL Lysis Buffer and 10 µL Proteinase K. Vortex for 30 seconds. Centrifuge briefly. Incubate at 65°C for 1 hour. (For samples containing 5% human albumin, add 10 µL of 5M NaCl, 200 µL Lysis Buffer and 20 µL Proteinase K. Vortex for 30 seconds. Centrifuge briefly. Then incubate at 30°C for 1 hour.)
    c) NOTE: The required volumes of Proteinase K and Lysis Buffer can be adjusted based on the sample's protein concentration, according to the following:

Sample protein concentration	Proteinase K	Lysis Buffer
0-50 mg/mL	10 µL	100 µL
50-100 mg/mL	20 µL	200 µL

2. Binding the DNA.
    a) Vortex and centrifuge briefly. Add 209.2 µL Working Binding Solution, 250 µL isopropanol and 20 µL Magnetic Beads.
    b) NOTE: Before use, the Magnetic Beads must be thoroughly suspended.
    c) Vortex for 10 minutes. Centrifuge briefly for 10 seconds.
    d) Place the tubes in the magnetic stand for 1 minute or until the beads are gathered.
    e) Remove the supernatant without disturbing the Magnetic Beads.
3. Wash the DNA.
    a) Add 500 µL Wash Buffer (with ethanol) to each tube. Vortex for 30 seconds. Fully disperse the Magnetic Beads to prevent any aggregation.
    b) Centrifuge for 10 seconds.
    c) Place the tubes in the magnetic stand for 1 minute or until the beads are gathered.
    d) Remove the supernatant without disturbing the Magnetic Beads.
    e) Add 500 µL fresh prepared 80% ethanol, vortex for 30 seconds to ensure Magnetic Beads are dispersed. Centrifuge for 10 seconds. Place the tubes in the magnetic stand with the pellet against the magnet. Then let the tubes stand for 1 minute. Remove and discard the supernatant.
    f) Centrifuge for 10 seconds. Place the tube on the magnetic stand and remove residual ethanol.
    g) Open the tube lids. Air dry the Magnetic Beads for 3-5 minutes at room temperature.
    h) NOTE: Residual ethanol will interfere with the subsequent PCR reaction. Please ensure that all the ethanol has completely evaporated. Do not over dry the magnetic beads, which will affect the recovery of nucleic acid.
4. Elute the DNA.
    a) Add 100µL Elution Buffer to each tube, vortex for 1min, then incubate the tubes at 70°C for 7 min. Vortex two or three times to resuspend beads.
    b) Centrifuge the tubes briefly for 1 minute with a high-speed centrifuge and place the tubes into the

magnetic stand with the pellet against the magnet. Then transfer eluate to a new 1.5mL tube.

c) NOTE: This kit can be used for automated DNA extraction

FAQs

How long can the eluted DNA be stored?

It is recommended to finish qPCR testing on the same cay as extraction or within 24 hours. The DNA can be temporarily stored at 2°C to 8°C.

Why am I getting an abnormal recovery?

Please see the table below for possible reasons and solutions for an abnormal recovery:

Possible Reasons	Actions to Take
Wash buffer doesn't have ethanol	Before first use, please add 30 mL 100% ethanol to wash buffer bottle.
80% ethanol is not fresh	Prepare new 80% ethanol for the experiment.
Isopropanol is not AR grade	Use AR grade isopropanol for the experiment.
Residual DNase	EDTA should be added to samples if Benzonase is used in purification process. Add 2μL 0.5m EDTA to 100 μL sample.
High protein concentration	Extend the digestion time with more Proteinase k and lysis buffer.
Residual enzyme	Pre-heat the sample at 95°C for 10 minutes.
Magnet is too dry	Air dry the magnetic beads for 3-5 minutes at room temperature.

RELATED PRODUCTS

Product Name	Catalog No.
Host Cell Residual DNA (magnetic beads) Sample Preparation Kit	DK-SP001
Mycoplasma DNA (qPCR) Detection Kit	DK-M002
E. coli Residual DNA (qPCR) Detection Kit	DK-E003
CHO Residual DNA (qPCR) Detection Kit	DK-C004
Plasmid DNA Residue (qPCR) Detection Kit	DK-P005