STDNA

Stool DNA Isolation Kit

Cat. No:

FPKT001.0025

FPKT001.0050

FPKT001.0100

Contents

Components	25 preps	50 preps	100 preps
STD1 Buffer	33 ml	33 ml * 2	33 ml*4
STD2 Buffer	8 ml	15 ml	30 ml
STD3 Buffer	5 ml	10 ml	20 ml
STD4 Buffer	7.5 ml	15 ml	30 ml
Wash Buffer	6 ml	12 ml	12 ml * 2
Elution Buffer	2 ml	2 ml	2 ml
RNase A 10mg/ml	50 µl	100 µl	200 µl
Column and collection tubes	25 each	50 each	100 each
Glass Beads	50 P	100 P	200 P

Kit storage:

⚠ This kit should be stored at room temperature.

⚠ **RNase A** should be stored at -20 °C.

If properly stored, all kit components are stable until the expiration date printed on the label.

⚠ After adding **absolute ethanol** to **STD3 and STD4 buffers**, Store them at +2 to +8°C.

Additional Equipment and Reagent required

•Absolute ethanol

• Isopropanol

• Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force

• Microcentrifuge tubes, 1.5 ml, sterile

Application

This kit provides a convenient and rapid method to isolate total DNA from fresh, frozen, and preserved stool samples. The purified DNA is of the highest quality and is fully compatible with all downstream applications such as PCR, qPCR, NGS, and microarrays since all humic acid substances and other PCR inhibitors are removed during the isolation process.

The procedure is optimized to achieve reliable results within **90 min**.

Handling Requirements and Safety Information

⚠ All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

⚠ STD3 and STD4 Buffers contain guanidinium hydrochloride which is an irritant.

⚠ Do not allow STD3 and STD4 Buffers to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.

⚠ If you spill the reagent, dilute the spill with water before wiping it up.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lot numbers.

⚠ Immediately after usage, close all bottles to avoid leakage, varying buffer concentrations, or buffer conditions.

⚠ After first opening store all bottles in an upright position.

⚠ Do not allow the STD3 Buffer and STD4 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce highly toxic gas.

⚠ Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, viruses, or nucleases. Use disposable pipets and nuclease-free pipet tips to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

FPKT001.0025-25 preps

⚠ **Add 5 ml absolute ethanol** to STD4 Buffer Bottle.

⚠ **Add 24 ml of absolute ethanol** to the Wash Buffer Bottle.

FPKT001.0050-50 preps

⚠ **Add 10 ml absolute ethanol** to STD4 Buffer Bottle.

⚠ **Add 48 ml of absolute ethanol** to the Wash Buffer Bottle.

FPKT001.0100-100 preps

⚠ **Add 20 ml absolute ethanol** to STD4 Buffer Bottle.

⚠ **Add 48 ml of absolute ethanol** to each Wash Buffer Bottle.

Please note that the Ethanol concentration of STD4 Buffer and Wash Buffer may decrease during long-term storage resulting in a drop-down of the final DNA yield.

⚠ before starting Incubate the **Elution buffer at 70 ° C** until the end of the protocol to obtain the maximum yields.

Protocol

1. Sample preparation
Add 250 mg of stool sample to a 2ml microtube.
And then Add 2 beads into the 2ml microtube.

⚠If the sample is dry, reduce the sample size to ≤ 50 mg.

⚠If the sample is liquid, add 200 µl 2ml microtube.

2. Add 1ml STD1 Buffer to the 2ml microtube. Vortex at maximum speed **for 2 minutes** (Make sure stool sample is homogenized completely). Incubate **at 70 °C for 15 min,** with gentle shaking by hand every 5 min.

3. Centrifuge at 4°C for 5min at 16,000×g. Transfer the supernatant to a new 2-ml microtube.

4. Add 300 µl STD1 Buffer to the microtube and Vortex at maximum speed **for 2 minutes.** Incubate **at 70 °C for 15 min.**

5. Centrifuge at 4°C for 5min at 16,000×g. Transfer the supernatant to a new 2-ml microtube.

6. Add 300 µl of STD2 Buffer to the sample. mix well, and incubate on ice for 5 min. Centrifuge at full speed (~16,000 x g) for 10 minutes.

7. Transfer 400 µl of the supernatant into a new 2ml microtube.

8. Add 2 µl of RNase A to the sample. mix well, and incubate **at 37 °C for 15 min.**
9. Add 200 µl STD3 Buffer and **200 µl Isopropanol** to the sample. mix well.

10. Transfer 800 µl of supernatant into a DNA spin column assembled in a clean collection tube (provided). Centrifuge at **10000×g for 1 min** at room temperature. Discard flow through.

11. Add 500 µl **STD4** to the spin column and centrifuge **at 10000×g for 1 min**. Discard flow through.

12. Add **700 µl wash buffer** to the spin column and **centrifuge (10000×g, 1 min).** Discard flow through.

13. Centrifuge the DNA spin column at **10000×g for 3 min** to remove residual ethanol.

14. Place the DNA spin column into the clean new microtube. Add 30 µl of pre-warmed Elution Buffer or sterile DDW directly onto the column membrane and stand for 1 min. Centrifuge at **10000×g for 1 min**.

15. Add another **30 µl pre-warmed Elution Buffer** or sterile DDW directly onto the column membrane and stand for 1min. Centrifuge at **10000×g for 1 min**.

16. Check **7 µl of extracted** DNA by 0.5 % agarose gel electrophoresis. **Store DNA at -20°C**.