DNA Purification from fecal samples

ISOSPIN Fecal DNA

Manual (Ver. 02)

Code No. 315-08621

NIPPON GENE CO., LTD.

I Introduction

The “ISOSPIN Fecal DNA” is a kit for DNA isolation from fecal sample.

II Components

Table 1 ISOSPIN Fecal DNA (Code No. 315-08621, 50 preps)

Component	Quantity	Storage
FE1 Buffer *1	35 ml	15-25°C
FE2 Buffer	4.5 ml	15-25°C
FB Buffer	40 ml	15-25°C
FW Buffer	40 ml	15-25°C
TE (pH8.0)	5 ml	15-25°C
RNase A (100 mg/ml)	0.5 ml	15-25°C (4°C or -20°C for long-term storage)
Beads Tube	50 tubes	15-25°C
Spin Column (Set of Spin Column and Collection Tube)	50 sets	15-25°C

*1 If precipitation is visible in FE1 Buffer, warm the buffer at 37-65°C and shake well to dissolve the precipitation.

III Storage

Room Temperature (15-25°C)

- RNase A is stable for longer periods when stored at 4°C or -20°C.
- Storage of FE1 Buffer at a low temperature may cause precipitation. If precipitation is visible in FE1 Buffer, warm the buffer at 37-65°C and shake well to dissolve the precipitation.

IV Precautions

- This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals.
- Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from Nippon Gene.

V Protocol

Perform the procedure (and the centrifugation) at room temperature (15-25°C), unless otherwise indicated.

Different protocols for DNA extraction.

1. Standard Protocol (Bead-beating)
2. Optional Protocol for DNA isolation from fecal suspension samples
3. Optional Protocol using heat block; alternative to Bead-beating method

Required materials not provided

- Isopropanol
- Bead homogenizer (available for 2 ml tube, 4-6m/s velocity)
- Micropipettes
- Micropipette tips
- 1.5 ml microcentrifuge tube
- Microcentrifuge capable of 13,000 × g
- Vortex mixer
- Heat block (optional protocol)
- 2.0 ml microcentrifuge tube (optional protocol)
- Wet ice if necessary

■ Standard Protocol (Bead-beating)

1. Weigh out 0.2 g of fresh or frozen fecal sample, then transfer the sample to a Beads Tube.
2. Add 700 µl of FE1 Buffer and 10 µl of RNase A.
3. Tighten the cap securely, then homogenize by bead beating for 30-45 seconds at 4-6 m/s or at 4,200-6,800 rpm.
4. Spin down briefly, add 90 µl of FE2 Buffer and mix thoroughly by vortexing.
   Note: If the Bead Tube is heated by bead beating, return it to room temperature before adding FE2 Buffer.
5. Centrifuge at 12,000 × g for 15 minutes.
6. Transfer up to 500 µl of the supernatant to a new 1.5 ml microcentrifuge tube, being careful not to disturb the bead pellet.
7. Add 200 µl of FB Buffer and 200 µl of isopropanol to 500 µl of the collected supernatant, and mix well by inverting.
   Note: The each volume of FB Buffer and isopropanol should be 0.4 times volume of collected supernatant.
8. Prepare a Spin Column (Set of Spin Column and Collection Tube) provided. Apply the mixture (maximum 900 µl) from step 7 to the Spin Column. Centrifuge at 13,000 × g for 30 seconds.
9. Discard flow-through, then place the Spin Column back into the same Collection Tube.
10. Apply 600 µl of FB Buffer to the Spin Column, and centrifuge at 13,000 × g for 1 minute.
11. Discard flow-through. Place the Spin Column back into the same Collection Tube.
12. Apply 600 µl of FW Buffer to the Spin Column, and centrifuge at 13,000 × g for 1 minute.
13. Discard flow-through and Collection Tube.
14. Place the Spin Column to a new 1.5 ml microcentrifuge tube.
15. Apply 50-100 µl of TE (pH8.0) near the center of the column membrane, and incubate at room temperature for 3 minutes.
    Note: Elution with 50 µl (instead of 100 µl) increases the final DNA concentration in the eluate.
16. Centrifuge at 13,000 × g for 1 minute.
17. Remove the column from the 1.5 ml microcentrifuge tube, and then close the lid of the tube. The purified DNA is in the tube.

■ Optional Protocol for DNA isolation from fecal suspension samples

1. Resuspend fecal suspension samples by vortexing or pipet repeatedly, and then transfer 200 µl of the sample into a Beads Tube.
2. Add 700 µl of FE1 Buffer and 10 µl of RNase A.
3. Tighten the cap securely, then homogenize by bead beating for 5 minutes at 4-6 m/s or at 4,200-6,800 rpm.

   Note: If the bead homogenizer cannot process for 5 minutes at one time, process the sample within the range of the time that homogenizer can process at one time. Then process repeatedly to be 5 minutes totally.
4. Spin down briefly, add 90 µl of FE2 Buffer and mix thoroughly by vortexing.

   Note: If the Bead Tube is heated by bead beating, return it to room temperature before adding FE2 Buffer.
5. Centrifuge at 12,000 × g for 15 minutes.
6. Transfer up to 600 µl of the supernatant to a new 1.5 ml microcentrifuge tube, being careful not to disturb the bead pellet.
7. Add 240 µl of FB Buffer and 240 µl of isopropanol to 600 µl of the collected supernatant, and mix well by inverting.

   Note: The each volume of FB Buffer and isopropanol should be 0.4 times volume of collected supernatant.
8. Prepare a Spin Column (Set of Spin Column and Collection Tube) provided. Apply the mixture (maximum 900 µl) from step 7 to the Spin Column. Centrifuge at 13,000 × g for 30 seconds.

   Note: If the mixture from step 7 is more than 900 µl, repeat step 8 and 9 to apply the remaining mixture
9. Discard flow-through, then place the Spin Column back into the same Collection Tube.
10. Perform wash steps with FB Buffer and FW buffer, and then elute DNA (refer to the steps 10-17 as described in the Standard Protocol).

■ Optional Protocol using heat block; alternative to Bead-beating method

1. Weigh out 0.2 g of fresh or frozen fecal sample, and then transfer the sample to a Beads Tube or a new 2.0 ml microcentrifuge tube.
2. Add 700 µl of FE1 Buffer and 10 µl of RNase A. Tighten the cap securely, then mix thoroughly by vortexing or inverting.
3. Incubate the tube at 65°C for 15 minutes in a heat block, and then mix well by vortexing or inverting. Repeat this step another three times (i.e., incubate at 65°C for totally 60 minutes).

   Note: Insufficient mixing will lead to low yield of DNA. Depending on the sample, it may not be homogeneous suspension after mixing. Make sure that the 65°C incubation time does not exceed 1 hour.
4. Incubate the tube at room temperature or on ice. Spin down briefly, add 90 µl of FE2 Buffer and mix thoroughly by vortexing.

   Note: Return it to room temperature before adding FE2 Buffer.
5. Centrifuge at 12,000 × g for 15 minutes.
6. Transfer up to 500 µl of the supernatant to a new 1.5 ml microcentrifuge tube, beking careful not to disturb the pellet.
7. Add 200 µl of FB Buffer and 200 µl of isopropanol to 500 µl of the collected supernatant, and mix well by inverting.

   Note: The each volume of FB Buffer and isopropanol should be 0.4 times volume of collected supernatant.
8. Prepare a Spin Column (Set of Spin Column and Collection Tube) provided. Apply the mixture (maximum 900 µl) from step 7 to the Spin Column. Centrifuge at 13,000 × g for 30 seconds.
9. Discard flow-through, then place the Spin Column back into the same Collection Tube.
10. Perform wash steps with FB Buffer and FW buffer, and then elute DNA (refer to the steps 10-17 as described in the Standard Protocol).

■ Quick protocol (Standard protocol)

Weigh 0.2 g of fecal sample, and place into a Beads Tube.

← Add 700 µl of FE1 Buffer.

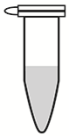
← Add 10 µl of RNase A, and tighten the cap securely.

Bead beating for 30-45 sec at 4-6 m/s or 4,200-6,800 rpm.

Spin down briefly.

← Add 90 µl of FE2 Buffer and mix thoroughly by vortexing.

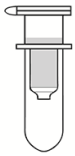
Centrifuge at 12,000 × g for 15 min.



Transfer 500 µl of the supernatant to a new 1.5 ml microcentrifuge tube.

← Add 200 µl of FB Buffer.

← Add 200 µl of isopropanol, and mix well by inverting.



Apply the mixture (maximum 900 µl) to a Spin Column.

Centrifuge at 13,000 × g for 30 sec.

Discard flow-through.

← Apply 600 µl of FB Buffer to the Spin Column.

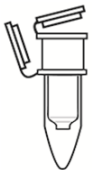
Centrifuge at 13,000 × g for 1 min.

Discard flow-through.

← Apply 600 µl of FW Buffer to the Spin Column.

Centrifuge at 13,000 × g for 1 min.

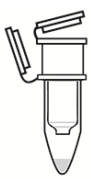
Discard flow-through and Collection Tube.



Place the Spin Column to a new 1.5 ml microcentrifuge tube.

← Apply 50 µl of TE (pH8.0) near the center of the column membrane.

Incubate at room temperature for 3 min.



Centrifuge at 13,000 × g for 1 min.

The purified DNA is in the tube.

If you have any questions, please contact Nippon Gene from www.nippongene.com.