www.real-biotech.com
Real Biotech Corporation
13F.-2, No.33, Sec. 1, Minsheng Rd., Banqiao City, Taipei County 220, Taiwan, R. O. C.
Tel: +886 2 2950 9000 Fax: +886 2 2950 0505

HiYield™ Genomic DNA Extraction Kit (Stool)

Cat. No.:	YGST50	YGST100
Product Name:	HiYield™ Genomic DNA Extraction Kit (Stool)	
Reactions:	50	100
Sample:	180-200mg of fresh or frozen stool samples	
Yield:	More than 5 µg of pure genomic DNA from 200 mg of stool samples	
Elution Volume:	30-100 µl	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 40 Minutes	

Description

HiYield™ Genomic DNA Extraction Kit (Stool) is optimized for rapid isolation of genomic DNA from microorganisms, such as bacteria, archaea, fungi, and algae in stool samples. The entire procedure can be completed in 40 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of more than 5 µg of pure genomic DNA from 200 mg of stool samples. Purified DNA, with approximately 20-30 kb, is suitable for direct use in PCR or other enzymatic reactions. Furthermore, the purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis.

Features

1. Reliable purification of high-quality genomic DNA within 40 minutes.
2. Consistent and high yields, with more than 5 µg of pure genomic DNA from 200 mg of stool samples.
3. Complete removal of all contaminants for sensitive downstream applications.
4. No phenol, chloroform or alcohol.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Quality Control

The quality of HiYield™ Genomic DNA Extraction Kit (Stool) is tested on a lot-to-lot basis by isolation of genomic DNA from 200 mg of stool samples. The purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 5 µg with A260/A280 ratio 1.7 - 2.0. The purified DNA is checked by electrophoresis.

Contents

Item	YGST50	YGST100
GST1 Buffer	50 ml	85 ml
GST2 Buffer	15 ml	30 ml
GST3 Buffer	90 ml	160 ml
Wash Buffer (concentrated)* (Add ethanol)	25 ml (100 ml)	25 ml (100 ml)
Elution Buffer	6 ml	30 ml
Inhibitor Removal Column	50 pcs	100 pcs
GD Column	50 pcs	100 pcs
Bead Tube	50 pcs	100 pcs
2 ml Centrifuge Tube	50 pcs	100 pcs
2 ml Collection Tube	50 pcs	100 pcs

* Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Safety Notes

GST3 Buffer contains chaotropic salts, during the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Storage

HiYield™ Genomic DNA Extraction Kit (Stool) shall be shipped and stored dry at room temperature (15-25℃).

Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:

1.5 ml microcentrifuge tubes, standard vortexer, absolute ethanol.

Caution: During operation, always wear a lab coat, disposable gloves and protective goggles.

Step 1 Sample Lysis	1. Transfer 180-220 mg of stool sample to a Bead Tube containing the ceramic beads. 2. Add 750 µl of GST1 Buffer to the Bead Tube containing the stool sample then vortex briefly. 3. Incubate the Bead Tube containing stool sample at 70ºC for 5 minutes. 4. Horizontally attach the Bead Tubes to a standard vortexer by taping or using an adapter. Vortex the Bead Tubes at maximum speed at room temperature for 10 minutes. 5. Centrifuge the Bead Tubes at 11,000 x g for 2 minutes at room temperature to eliminate the foam caused by detergents. Note: Preheat the required Elution Buffer (100 µl per sample) to 70ºC for DNA elution.
Step 2 PCR Inhibitors Removal	1. Transfer 600 µl of supernatant to a new 1.5 ml microcentrifuge tube (not provided). 2. Add 150 µl of GST2 Buffer to the microcentrifuge tube and vortex for 5 seconds. 3. Incubate the microcentrifuge tube at 0-4ºC for 5 minutes. 4. Centrifuge the microcentrifuge tube at 11,000 x g for 1 minute at room temperature to precipitate insoluble particles and PCR inhibitors. 5. Place an Inhibitor Removal Column (blue ring) in a 2 ml Centrifuge Tube. Transfer 500-600 µl of clear supernatant from the microcentrifuge tube to the Inhibitor Removal Column. 6. Centrifuge at 11,000 x g for 1 minute at room temperature then discard the Inhibitor Removal Column. Save the flow-through in the 2 ml Centrifuge Tube for DNA Binding. Note: If a pellet is in the flow-through, transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (not provided).

Step 3 DNA Binding	1. Add 900 µl of GST3 Buffer to the flow-through then mix IMMEDIATELY by shaking vigorously for 5 seconds. 2. Place a GD Column (green ring) in a 2 ml Collection Tube. Transfer 750 µl of sample mixture to the GD Column, centrifuge at 11,000 x g for 1 minute at room temperature then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Transfer the remaining sample mixture to the GD Column and centrifuge at 11,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.
Step 4 Wash	1. Add 400 µl of GST3 Buffer to the GD Column. Centrifuge at 11,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GD Column. Centrifuge at 11,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. 3. Repeat to add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GD Column and centrifuge again. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. 4. Centrifuge at 11,000 x g for 3 minutes at room temperature to dry the column matrix.
Step 5 DNA Elution	1. Transfer the dried GD Column to a new 1.5 ml microcentrifuge tube. 2. Add 30-100 µl of pre-heated Elution Buffer[1], TE Buffer[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 11,000 x g for 2 minutes at room temperature to elute the purified DNA. NOTE: [1] If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer. [2] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. [3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Problem	Possible Reasons / Solution
Low Nucleic Acid Yield	**Too much starting materials** Too much stool sample was added to the Bead Tube. Too little space in the Bead Tube results in the beads cannot efficiently disrupt the sample. **Sample lysis or homogenization was incomplete** Horizontally vortex the Bead Tube at the maximum speed using a vortexer at room temperature for 10 minutes or using a Disruptor Genie or similar. **Incorrect DNA elution step** Pre-heat the Elution Buffer to 70 ºC prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely. Ensure to use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. **Inappropriate buffer preparation** Add appropriate volume of absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to use. If precipitates have formed in GST1 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.
Degraded DNA	**Mechanical sample disruption is too strong** Using alternative lysis method for less DNA shearing: After adding GST1 Buffer, vortex the Bead Tube at maximum speed for 5 seconds then incubate the Bead Tube at 70 ºC for 5 minutes. Repeat these steps for 3 times. This lysis method will reduce DNA shearing but may also reduce DNA yield.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** Following the wash step, dry the GD Column with additional centrifugation at 11,000 x g for 5 minutes to remove residual ethanol. **PCR inhibitors contamination** Using diluted DNA (1:10) as template to reduce the concentration of PCR inhibitors for PCR reactions. The DNA can be further purified using our HiYield Plus™ PCR Purification Kit (cat. no. QPP100) to eliminate PCR inhibitors.