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HiYield™ Genomic DNA Mini Kit (Bacteria)

Cat. No.:	YGC100	YGC300
Product Name:	HiYield™ Genomic DNA Mini Kit (Bacteria)	
Reactions:	100	300
Sample:	Up to 1 x 10^9 of Gram-negative and Gram-positive bacteria	
Yield:	1 x 10^9 Escherichia coli: up to 40 µg, 1 x 10^9 Bacillus subtilis: up to 15 µg	
Elution Volume:	30-200 µl	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 20 Minutes	

Description

HiYield™ Genomic DNA Mini Kit (Bacteria) is optimized for genomic and viral DNA purification from Gram-negative and Gram-positive bacterial cells. The entire procedure can be completed in 20 minutes without phenol/chloroform extraction or alcohol precipitation, with DNA yield up to 40 µg. Purified DNA is suitable for direct use in PCR or other enzymatic reactions. Furthermore, the purified DNA performs well in sensitive downstream analyses, such as quantitative PCR.

Features

1. Reliable purification of high-quality genomic DNA within 20 minutes.
2. Consistent and high yields, with DNA yield of up to 40 µg from 1 x 10^9 Escherichia coli.
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, Restriction Enzyme Digestions, Sequencing.

Quality Control

The quality of HiYield™ Genomic DNA Mini Kit (Bacteria) is tested on a lot-to-lot basis by isolation of genomic DNA from 1×10^9 of Escherichia coli culture harvested by centrifugation at 16,000 x g for 1 minute. 10 µl from a 50 µl eluate of purified DNA is analyzed by electrophoresis on a 1% agarose gel. The purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 25 µg with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Contents

Item	YGC100	YGC300
GPT Buffer*	30 ml	75 ml
GNT Buffer	30 ml	75 ml
GB Buffer	40 ml	100 ml
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)**	25 ml	50 ml
Elution Buffer	30 ml	75 ml
GD Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

*When extracting genomic DNA from Gram-positive bacteria, add lysozyme to GPT Buffer immediately prior to use. Once lysozyme is mixed with GPT Buffer, the mixture can be stored for 2 weeks at 4ºC. Lysozyme (20mg/100mg/500mg/1g) can be purchased directly from Real Biotech Corp.

** 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 to close the bottle tightly after each use to avoid ethanol evaporation.

Storage

HiYield™ Genomic DNA Mini Kit (Bacteria) shall be shipped and stored dry at room temperature (15-25℃). With proper storage, HiYield™ Genomic DNA Mini Kit (Bacteria) can be stored for up to 12 months without showing any deduction in performance and quality.

Bacteria Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. When extracting genomic DNA from Gram-positive bacteria, add lysozyme to GPT Buffer immediately prior to use. Once lysozyme is mixed with GPT Buffer, the mixture can be stored for 2 weeks at 4ºC. Lysozyme (20mg/100mg/500mg/1g) can be purchased directly from Real Biotech Corp.
2. 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 to close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:

Absolute ethanol, RNase-free 1.5 ml microcentrifuge tubes, pipette tips, 15 ml centrifuge tube (Gram -positive bacteria only), lysozyme (Gram-positive bacteria only).

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

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

Step 1 Sample Preparation	**Cell Harvesting for Gram-Negative Bacteria** 1. Transfer cultured bacterial cells (up to 1 x10^9) to a 1.5ml microcentrifuge tube. 2. Centrifuge at 14,000-16,000 x g for 1 minute and then discard the supernatant. 3. Add 200 µl of GNT Buffer to the tube and resuspend the cell pellet by vortexing or pipetting. 4. Incubate at room temperature for 5 minutes. Proceed to Step 2 Cell Lysis Below.
	Cell Harvesting for Gram-Positive Bacteria 1. Transfer cultured bacterial cells (up to 1 x10^9) to a 1.5ml microcentrifuge tube. 2. Centrifuge at 14,000-16,000 x g for 1 minute and then discard the supernatant. 3. Transfer the required volume of GPT (200 µl/sample) to a 15 ml centrifuge tube. Add lysozyme (20 mg/ml) to GPT Buffer (in the 15 ml centrifuge tube) then vortex to completely dissolve the lysozyme. 4. Transfer 200 µl of GPT Buffer (make sure lysozyme was added) to the sample in the 1.5 ml microcentrifuge tube then re-suspend the pellet by vortexing or pipetting. 5. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes. Proceed to Step 2 Cell Lysis Below.
Step 2 Cell Lysis	1. Add 200 µl of GB Buffer to the sample and mix by shaking vigorously for 5 seconds. 2. Incubate at 60ºC for 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution). (Optional) RNA Removal Step: Following 60ºC incubation, add 5 µl of RNase A (50 mg/ml) to the clear sample lysate and then mix by vortex. Incubate at room temperature for 5 minutes.
Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 2 minutes. Discard the 2 ml Collection Tube containing the flow-through then place the GD Column in a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] 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 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA concentration is required, repeat the elution step using the eluate only. If higher DNA yield is required, repeat the DNA elution step to increase DNA recovery and make the total elution volume to approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3]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. [4]If using water for elution, ensure the water pH is between 7.0 and 8.5. 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 Yield	**Incomplete buffer preparation** 1. When extracting genomic DNA from Gram-positive bacteria, add lysozyme to GPT Buffer immediately prior to use. Once lysozyme is mixed with GPT Buffer, the mixture can be stored for 2 weeks at 4ºC. Lysozyme(20mg/100mg/500mg/1g) can be purchased directly from Real Biotech Corp. 2. 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. **Incomplete cell lysis** Reduce the amount of starting material or separate the sample into multiple tubes. Make sure bacteria cells were completely homogenized in GNT Buffer or GPT Buffer. If extracting genomic DNA from Gram-positive bacteria, make sure lysozyme is added to GPT Buffer prior to use. **Incorrect DNA elution step** 1. Ensure that Elution Buffer, TE or water is added into the CENTER of the GD Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. 2. Repeating the elution step will increase yield. Repeating the elution step using the eluate only will increase DNA concentration.
Eluted DNA Does Not Perform Well In Downstream Applications	**Residual Ethanol Contamination** Following the wash step, dry the GD Column with additional centrifugation at 14,000-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.