

Reagent Genomic DNA Kit

For research use only

Sample : fresh blood, cultured animal cells, bacteria, fungus and tissue

Applications : PCR, AFLP, RFLP/PADP, Southern Blotting, Real-time PCR

Geneaid



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Introduction

The Reagent Genomic DNA Kit was designed specifically for DNA isolation from large volumes of blood. However, this highly versatile reagent is able to extract genomic DNA from various other samples, such as cultured cells, bacteria, fungus and tissue. RBC Lysis Buffer is included with the kit to remove non-nucleated red blood cells and to reduce hemoglobin contamination. When the blood sample is less than 50 µl or when using nucleated red blood cell samples, the Cultured Cells Protocol is recommended. If a larger sample size is required, the buffer volume can be scaled proportionately.

Quality Control

The quality of the Reagent Genomic DNA Kit is tested on a lot-to-lot basis by isolating genomic DNA from 300 µl of fresh whole human blood. The isolated DNA (5-15 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	GE003	GE100	GE01K
RBC Lysis Buffer	12 ml	360 ml	1000 ml x 3 500 ml x 1
Cell Lysis Buffer	3 ml	100 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml

Order Information

Product Name	Reactions	Cat. No.
Reagent Genomic DNA Kit	100/1000 rxns (1 ml of blood/rxn)	GE100/01K
Tri-Plant Genomic DNA Reagent Kit	100 rxns	GR100
Tri-RNA Isolation Reagent	100 rxns	RAR100
Tri-Plant RNA Isolation Reagent	100 rxns	RPR100
Tri-Total Nucleic Acid Isolation Reagent	100 rxns	NR100
96-Well Tri-Nucleic Acid Reagent Kit	2/4/10 x 96 rxns	NRP02/04/10
96-Well Tri-Plant Genomic DNA Reagent Kit	2/4/10 x 96 rxns	GRP02/04/10

Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Reagent Genomic DNA Kit Blood Protocol (50-300 µl)

- Additional requirements: microcentrifuge tubes, Isopropanol, absolute ethanol, RNase A (10 mg/ml)

Step 1 RBC Lysis	<ul style="list-style-type: none"> Collect fresh blood in EDTA-NA₂-treated (or other anticoagulant) collection tubes. Add up to 300 µl of blood into a 1.5 ml microcentrifuge tube. If the blood sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex. Incubate the tube for 5 minutes at room temperature. Centrifuge at 3,000 x g for 5 minutes. Remove the supernatant, but retain approximately 50 µl of residual buffer to resuspend the white cell pellet by vortex.
Step 2 Cell Lysis	<ul style="list-style-type: none"> Add 300 µl of Cell Lysis Buffer to the tube and mix by vortex. Incubate at 60°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. <hr/> <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> Add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.
Step 3 Protein Removal	<ul style="list-style-type: none"> Add 100 µl of Protein Removal Buffer to the sample lysate and vortex immediately for 10 seconds. Incubate on ice for 5 minutes. Centrifuge at 14-16,000 x g for 3 minutes.
Step 4 DNA Precipitation	<ul style="list-style-type: none"> Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube. Add 300 µl of Isopropanol and mix well by inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes. Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes. Discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Rehydration	<ul style="list-style-type: none"> Add 50-100 µl of TE Buffer or water and incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Reagent Genomic DNA Kit Blood Protocol (300 µl-3 ml)

- Additional requirements: 15 ml centrifuge tube, Isopropanol, absolute ethanol, RNase A (10 mg/ml)

Step 1 RBC Lysis	<ul style="list-style-type: none"> Collect fresh blood in EDTA-NA₂ treated (or other anticoagulant) collection tubes. Add the blood sample (up to 3 ml) to a 15 ml centrifuge tube. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex. Incubate the tube for 5 minutes at room temperature. Centrifuge at 3,000 x g for 5 minutes. Remove the supernatant, but retain approximately 300 µl of residual buffer to resuspend the white cell pellet by vortex.
Step 2 Cell Lysis	<ul style="list-style-type: none"> Add 3 ml of Cell Lysis Buffer to the tube and mix by vortex. Incubate at 60°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. <hr/> <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> Add 10 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 10 minutes.
Step 3 Protein Removal	<ul style="list-style-type: none"> Add 1 ml of Protein Removal Buffer to the sample lysate and vortex immediately for 10 seconds. Incubate on ice for 5 minutes. Centrifuge at 14-16,000 x g for 5 minutes.
Step 4 DNA Precipitation	<ul style="list-style-type: none"> Transfer the supernatant (approximately 4 ml) from Step 3 to a 15 ml centrifuge tube. Add 3 ml of Isopropanol and mix well by inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes. Carefully remove the supernatant and add 3 ml of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes. Discard the supernatant and air-dry the pellet for 20 minutes.
Step 5 DNA Rehydration	<ul style="list-style-type: none"> Add 100-300 µl of TE Buffer or water and incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Reagent Genomic DNA Kit Cultured Cell Protocol

- Additional requirements: microcentrifuge tubes, Isopropanol, absolute ethanol, RNase A (10 mg/ml)

Step1 Sample Preparation	<p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none"> ● Transfer cells (up to 1×10^7) to a 1.5 ml microcentrifuge tube and harvest with centrifugation for 20 seconds at 6,000 x g. ● Remove the supernatant, retaining approximately 50 μl of residual buffer to resuspend the white cell pellet by vortex.
Step 2 Cell Lysis	<ul style="list-style-type: none"> ● Add 300 μl of Cell Lysis Buffer to the sample and mix by vortex. ● Incubate at 60°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. <hr/> <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> ● Add 5 μl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ● Incubate at room temperature for 5 minutes.
Step 3 Protein Removal	<ul style="list-style-type: none"> ● Add 100 μl of Protein Removal Buffer to the sample lysate and vortex immediately for 10 seconds. ● Incubate on ice for 5 minutes. ● Centrifuge at 14-16,000 x g for 3 minutes.
Step 4 DNA Precipitation	<ul style="list-style-type: none"> ● Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube. ● Add 300 μl of Isopropanol and mix well by inversion. ● Centrifuge at 14-16,000 x g for 5 minutes. ● Discard the supernatant and add 300 μl of 70% ethanol to wash the pellet. ● Centrifuge at 14-16,000 x g for 3 minutes. ● Discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Rehydration	<ul style="list-style-type: none"> ● Add 50-100 μl of TE Buffer and incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Reagent Genomic DNA Kit Gram-positive Bacteria Protocol

- If the sample is gram-negative bacteria, use the Cultured Cell Protocol.
- Additional requirements: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0, **prepare the lysozyme buffer immediately prior to use**).

Step1 Cell Harvesting/ Pre-Lysis	<p>Gram-positive bacteria</p> <p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none"> ● Transfer the bacterial culture (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ● Centrifuge for 1 minute at 14-16,000 x g and discard the supernatant. ● Add 100 μl of lysozyme buffer to the tube and resuspend the cell pellet by vortex or pipetting. ● Incubate at room temperature for 20 minutes. During incubation, invert the tube every 2-3 minutes. ● Proceed with Step 2 (Cell Lysis) of the Cultured Cell Protocol.
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Reagent Genomic DNA Kit Fungus Protocol

- Additional requirements: sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol), lyticase or zymolase.

Step1 Cell Harvesting/ Pre-Lysis	<ul style="list-style-type: none"> ● Harvest fungus cells (up to 5×10^7) by centrifugation for 10 minutes at 5,000 x g. ● Resuspend the pellet in 600 μl of sorbitol buffer. ● Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes. ● Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. ● Proceed with Step 2 (Cell Lysis) of the Cultured Cell Protocol.
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Reagent Genomic DNA Kit Tissue Protocol

- Additional requirements: micropestle, Proteinase K (10 mg/ml), microcentrifuge tubes, Isopropanol, absolute ethanol, RNase A (10 mg/ml)

Step 1 Tissue Dissociation	<ul style="list-style-type: none"> ● Cut up to 20 mg of animal tissue (or 0.5 cm of mouse tail) and transfer to a 1.5 ml microcentrifuge tube. ● Use a micropestle to grind the tissue a few times. ● Add 300 µl of Cell Lysis Buffer to the tube and continually homogenize the sample tissue with grinding.
Step 2 Cell Lysis	<ul style="list-style-type: none"> ● Add 20 µl of Proteinase K to the sample mixture and mix by vortex. ● Incubate at 60°C for 30-60 minutes to lyse the sample until the tissue particulates have dissolved. During incubation, invert the tube every 5 minutes. <hr/> <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> ● After 60°C incubation, add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ● Incubate at room temperature for 5 minutes.
Step 3 Protein Removal	<ul style="list-style-type: none"> ● Add 100 µl of Protein Removal Buffer to the sample lysate and vortex immediately for 10 seconds. ● Incubate on ice for 5 minutes. ● Centrifuge at 14-16,000 x g for 3 minutes.
Step 4 DNA Precipitation	<ul style="list-style-type: none"> ● Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube. ● Add 300 µl of Isopropanol and mix well by inverting. ● Centrifuge at 14-16,000 x g for 5 minutes. ● Discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. ● Centrifuge at 14-16,000 x g for 3 minutes. ● Discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Rehydration	<ul style="list-style-type: none"> ● Add 50-100 µl of TE buffer and incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

Reagent Genomic DNA Kit Paraffin Embedded Tissue

- Additional requirements: microcentrifuge tube, xylene, absolute ethanol

Step 1 Sample Preparation	<ul style="list-style-type: none"> ● Slice small sections (up to 20 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube. ● Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation. ● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. ● Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. ● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. ● Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting. ● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. ● Open the tube and incubate at 37°C for 15 minutes to evaporate the ethanol residue. ● Proceed to Step 1 (Tissue Dissociation) of the Tissue Protocol.
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Troubleshooting

Problem	Possible Reasons/Solution
Incomplete Lysis	<p>Too much sample was used</p> <ul style="list-style-type: none"> ● Reduce the sample volume or separate it into multiple tubes.
Low Yield	<p>Precipitate was formed at Step 4</p> <ul style="list-style-type: none"> ● Reduce the sample material. ● Increase standing time to improve DNA precipitation.