



# HiYield™ Genomic DNA Extraction Kit Reagent

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### **Genomic DNA Extraction Kit**

#### **Blood**

Cat.No. YGBE1KR // YGBE 100R

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### **Genomic DNA Extraction Kit**

#### **Cultured Cells /Tissue / Bacterial / Yeast**

Cat.No. YGE100

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### **Genomic DNA Extraction Kit (Reagent)**

**Blood**

**Cat.No. YGBE1KR // YGBE 100R**

Store at room  
temperature  
15 °C ~ 25 °C

### **Kit Contents**

**Cat.No. YGBE1KR**

RBC Lysis Buffer.....1000 ml X 3  
Cell Lysis Buffer.....1000 ml X 1  
Protein Precipitation Buffer.....350 ml X 1  
DNA Hydration Buffer.....500 ml X 1  
RNase A.....5 ml X 1

**Cat.No. YGBE 100R**

RBC Lysis Buffer.....180 ml X 2  
Cell Lysis Buffer.....100 ml X 1  
Protein Precipitation Buffer.....40 ml X 1  
DNA Hydration Buffer.....50 ml X 1  
RNase A.....500 µl X 1

**Sample Source :** Fresh human blood, Bacteria, Tissue, Cultured cells, Yeast.

**Yield :** Dependent on sample size and type.

**Operation time :** 30 mins

#### **Additional Requirements:**

Microcentrifuge Tube, Isopropanol, 70% Ethanol.

## Description

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Genomic DNA Extraction kit (Reagent) is designed to purify genomic DNA from 50 µl to 10 ml of Blood. This kit can also be used to purify genomic DNA from cultured cells, yeast and bacteria. Whole blood samples are first treated with RBC lysis buffer to remove RBC. The included cell lysis buffer efficiently lyses nucleated and cultured cells. Protein is removed from the lysate with the protein removal buffer. Purified DNA can be utilized for applications such as enzyme digestion, human genetic testing and PCR.

## Applications

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PCR, Southern blotting, RADP/ AFLP

## Quality Control

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The quality of Genomic DNA Extraction Kit (Reagent) is tested on a lot-to-lot basis. The kits are tested by isolation of genomic DNA from 1 ml of human whole blood. Purified DNA is quantified with by spectrophotometry. Yield of genomic DNA is more than 10 µg with A260/A280 ratio 1.6 to 1.8

## Protocols Included

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<i>Blood (Sample Volumes: 50 µl-300µl)</i>	<i>Blood (Sample Volumes: 300 µl- 3 ml)</i>
<i>Blood (Sample Volumes:3ml-10ml)</i>	<i>Cultured Animal Cells</i>
<i>Animal or insect tissue (&lt; 25 mg), spleen (&lt; 10 mg), mouse tail (&lt; 1.5 cm)</i>	<i>Gram-Negative Bacterial &lt;10<sup>9</sup></i>
<i>Gram-Positive Bacterial &lt;10<sup>9</sup></i>	<i>Yeast</i>

## Reference

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Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

## Protocol Fresh Blood 50-300 $\mu$ l

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### **Additional requirements**

1.5ml microcentrifuge tube, 15ml centrifuge tube.

Isopropanol.

70% Ethanol.

### ▼ RBC Lysis

1. Collect fresh blood in EDTA-NA2-treated collection tube (or other anticoagulants).
2. Apply up to 300  $\mu$ l blood to a 1.5ml microcentrifuge tube. If blood sample is more than 300  $\mu$ l blood (up to 1 ml), apply the blood sample to a sterile 15ml centrifuge tube.
3. Add 3 times sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate for 5 min at room temperature.
5. Centrifuge at 3,000  $\times$  g for 5 minutes.
6. Remove the supernatant, but retain about 50  $\mu$ l residual buffer to resuspend the white cell pellet by vortexing.

### ▼ Cell Lysis

7. Add 300  $\mu$ l Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60 $^{\circ}$ C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

### **Optional Step: RNA Degradation**

*If RNA-free genomic DNA is required, perform the optional step.*

*a. Add 10  $\mu$ l of RNase A (10 mg/ ml) to sample lysate and mix by vortexing.*

*b. Incubate at room temperature for 10 minutes.*

### ▼ Protein Remove

9. Add 100  $\mu$ l Protein Precipitation Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed 10,000  $\times$  g (13,000 rpm) for 5 minutes.

### ▼ DNA Precipitation

12. Transfer the supernatant from Step 11 to a 1.5 ml microcentrifuge tube.
13. Add 300  $\mu$ l Isopropanol and mix well by inverting.
14. Centrifuge at full speed 10,000  $\times$  g (13,000 rpm) for 5 minutes.
15. Discard the supernatant and add 300  $\mu$ l of 70% ethanol to wash the pellet.
16. Centrifuge at full speed 10,000  $\times$  g (13,000 rpm) for 5 minutes.
17. Discard the supernatant and air-dry the pellet for 10 minutes.

## Protocol Fresh Blood 50-300µl ( continued )

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### ▼ DNA Rehydration

18. Add 50-100 µl of DNA Hydration Buffer and incubate at 60°C for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

## Protocol Fresh Blood 300µl-3ml

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### **Additional requirements**

- 1.5ml microcentrifuge tube, 15ml centrifuge tube.*
- Isopropanol.*
- 70% Ethanol.*

### ▼ RBC Lysis

1. Collect fresh blood in EDTA-NA2-treated collection tube (or other anticoagulants).
2. Apply blood sample (up to 3 ml) to a sterile 15 ml centrifuge tube.
3. Add 3 times sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate for 5 min at room temperature.
5. Centrifuge at 2,000 × g for 5 minutes.
6. Remove the supernatant, but retain about 300 µl residual buffer to resuspend the white cell pellet by vortexing.

### ▼ Cell Lysis

7. Add 3ml Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60°C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

### **Optional Step: RNA Degradation**

*If RNA-free genomic DNA is required, perform the optional step.*

- a. Add 10 µl of RNase A (10 mg/ ml) to sample lysate and mix by vortexing.*
- b. Incubate at room temperature for 10 minutes.*

### ▼ Protein Remove

9. Add 1ml Protein Precipitation Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed 2,000 x g for 5 minutes.

## Protocol Fresh Blood 300µl-3ml ( continued )

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### ▼ DNA Precipitation

12. Transfer supernatant from Step 11 to a 15 ml centrifuge tube.
13. Add 3ml Isopropanol and mix well by inverting.
14. Centrifuge at full speed 2,000 x g for 5 minutes.
15. Carefully remove the supernatant and add 3 ml of 70% ethanol to wash the pellet.
16. Centrifuge at full speed 2,000 x g for 5 minutes.
17. Discard the supernatant and air-dry the pellet for 20 minutes.

### ▼ DNA Rehydration

18. Add 100-300 µl of DNA Hydration Buffer and incubate at 60°C for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

## Protocol Fresh Blood 3-10ml

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### **Additional requirements**

*50ml microcentrifuge tube.*

*Isopropanol.*

*70% Ethanol.*

### ▼ RBC Lysis

1. Collect fresh blood in EDTA-NA2-treated collection tube (or other anticoagulants).
2. Apply blood sample (up to 10 ml) to a sterile 50 ml centrifuge tube.
3. Add 3 times sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate for 5 min at room temperature.
5. Centrifuge at 2,000 × g for 5 mins.
6. Remove the supernatant, but retain about 500 µl residual buffer to resuspend the white cell pellet by vortexing.

### ▼ Cell Lysis

7. Add 10ml Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60°C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

## Protocol Fresh Blood 3-10ml ( continued )

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### **Optional Step: RNA Degradation**

*If RNA-free genomic DNA is required, perform the optional step.*

- a. Add 10  $\mu$ l of RNase A (10 mg/ ml) to sample lysate and mix by vortexing.
- b. Incubate at room temperature for 10 minutes.

### ▼ **Protein Remove**

9. Add 3.3ml Protein Precipitation Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed for 5 minutes.

### ▼ **DNA Precipitation**

12. Transfer supernatant (about 4 ml) from Step 11 to a 50 ml centrifuge tube.
13. Add 10ml Isopropanol and mix well by inverting.
14. Centrifuge at full speed 2,000 x g for 5 minutes.
15. Discard the supernatant and add 10ml 70% ethanol to wash the pellet.
16. Centrifuge at full speed 2,000 x g for 5 minutes.
17. Discard the supernatant and air-dry the pellet for 30 minutes.

### ▼ **DNA Rehydration**

18. Add 300-600  $\mu$ l of DNA Hydration Buffer and incubate at 60oC for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

# Genomic DNA Extraction Kit (Reagent)

Cultured Cells / Tissue / Bacterial / Yeast  
Cat.No. YGE 100

Store at room  
temperature  
15°C~25°C

## Kit Contents

Cat.No. YGE100

Cell Lysis Buffer.....	100 ml X 1
Protein Precipitation Buffer.....	40 ml X 1
DNA Hydration Buffer.....	50 ml X 1
RNase A.....	500 µl X 1
Proteinase K *	11 mg X 2

**Sample Source :** Fresh human blood, Bacteria, Tissue, Cultured cells, Yeast.

**Yield :** Dependent on sample size and type.

**Operation time :** 30 mins

### Additional Requirements:

Microcentrifuge Tube, Isopropanol, 70% Ethanol.

\* Add 1.1 ml ddH<sub>2</sub>O to the tube and mix by vortexing. Store prepared Proteinase K (10mg/ml) at 4 °C. For long term storage , aliquot and store at -20 °C.

### **Additional requirements**

1.5ml microcentrifuge tube

Isopropanol

70% Ethanol

### **Sample Preparation**

#### **▲ Cell Cultured**

##### **Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)**

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

##### **Suspension Cultured Animal Cells**

Transfer cells ( $3-5 \times 10^6$ ) to a 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50  $\mu$ l of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

Add 600  $\mu$ l of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes, and proceed to **Protein Remove Step 3 ( page 10 )**.

#### **▲ Tissue**

*Animal or insect tissue (< 25 mg), spleen (< 10 mg), mouse tail (< 1.5 cm)*

1. Transfer tissue to a 1.5 ml microcentrifuge tube and use a micropestle to grind the tissue a few times. Add 600  $\mu$ l of Cell Lysis Buffer to the tube and continue to homogenize the sample tissue with grinding.
2. Add 20  $\mu$ l of Proteinase K to the tube then mix by vortex. Incubate at 60°C for 30-60 minutes or until the tissue has dissolved completely. During incubation, invert the tube periodically and proceed to **Protein Remove Step 3 ( page 10 )**.

### ▲ Bacteria

#### ***Additional requirements***

*Lysozyme Buffer*

*(20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0)*

***For Gram-Negative bacterial sample, use Cultured Cells Protocol.***

1. Transfer bacterial culture ( $< 10^9$ ) to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard the supernatant.
3. Add 100  $\mu$ l of Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 20 minutes. During incubation, invert the tube every 2-3 min.
5. Proceed with **DNA Precipitation Step 7 ( page 10 )**.

### ▲ Yeast

#### ***Additional requirements***

*Lysozyme Buffer*

*(20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0)*

1. Harvest yeast cells (up to  $5 \times 10^7$ ) by centrifugation for 10 min at 5,000x g.
2. Resuspend the pellet in 600  $\mu$ l sorbitol buffer.
3. Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 min.
4. Centrifuge the mixture for 10 min at 2,000g to harvest Spheroplast.
5. Proceed with **DNA Precipitation Step 7 ( page 10 )**.

### ▼ Cell Lysis

1. Add 300  $\mu$ l Cell Lysis Buffer to the tube and mix by vortexing.
2. Incubate at 60°C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

### **Optional Step: RNA Degradation**

*If RNA-free genomic DNA is required, perform the optional step.*

- a. Add 10  $\mu$ l of RNase A (10 mg/ml) to sample lysate and mix by vortexing.
- b. Incubate at room temperature for 10 minutes.

### ▼ Protein Remove

3. Add 100  $\mu$ l Protein Precipitation Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
4. Incubate on ice for 5 minutes.
5. Centrifuge at full speed 10,000 x g (13,000 rpm) for 5 minutes.

### ▼ DNA Precipitation

6. Transfer supernatant from Step 5 to a 1.5 ml microcentrifuge tube.
- 7 Add 3/4 times volume of Isopropanol and mix well by inverting.
8. Centrifuge at full speed 10,000 x g (13,000 rpm) for 5 minutes.
9. Carefully remove the supernatant and add 300  $\mu$ l 70% ethanol to wash the pellet.
10. Centrifuge at full speed 10,000 x g (13,000 rpm) for 5 minutes.
11. Discard the supernatant and air-dry the pellet for 10 minutes.

### ▼ DNA Rehydration

12. Add 50-100  $\mu$ l of DNA Hydration Buffer and incubate at 60°C for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

## Troubleshooting

Problem	Possible Reason/Solution
<b>Improper sample homogenization</b>	Yield and quality of DNA will be higher when fresh samples or samples which have been flash frozen and stored at -20°C or -70°C are used. Fresh blood is recommended. However, frozen or blood treated with anticoagulants (EDTA etc.) can also be used. Increased storage length decreases DNA yield.
<b>Incomplete protein removal</b>	A solid protein pellet must be formed following centrifugation in <b>Protein Remove Step</b> .
<b>RNA contamination</b>	Perform the optional <b>RNA Degradation step</b> .
<b>Slow DNA rehydration</b>	In <b>DNA Rehydration step</b> , tap the bottom of the tube occasionally to facilitate DNA rehydration. If the DNA pellet is too dry, incubate at 60°C for 60 minutes or at room temperature overnight.
<b>Eluted DNA does not perform well in downstream applications</b>	Increase DNA pellet drying time to ensure residual ethanol is completely evaporated