

QuickGene Series **Application Guide**

Genomic DNA Isolation from Human Whole Blood

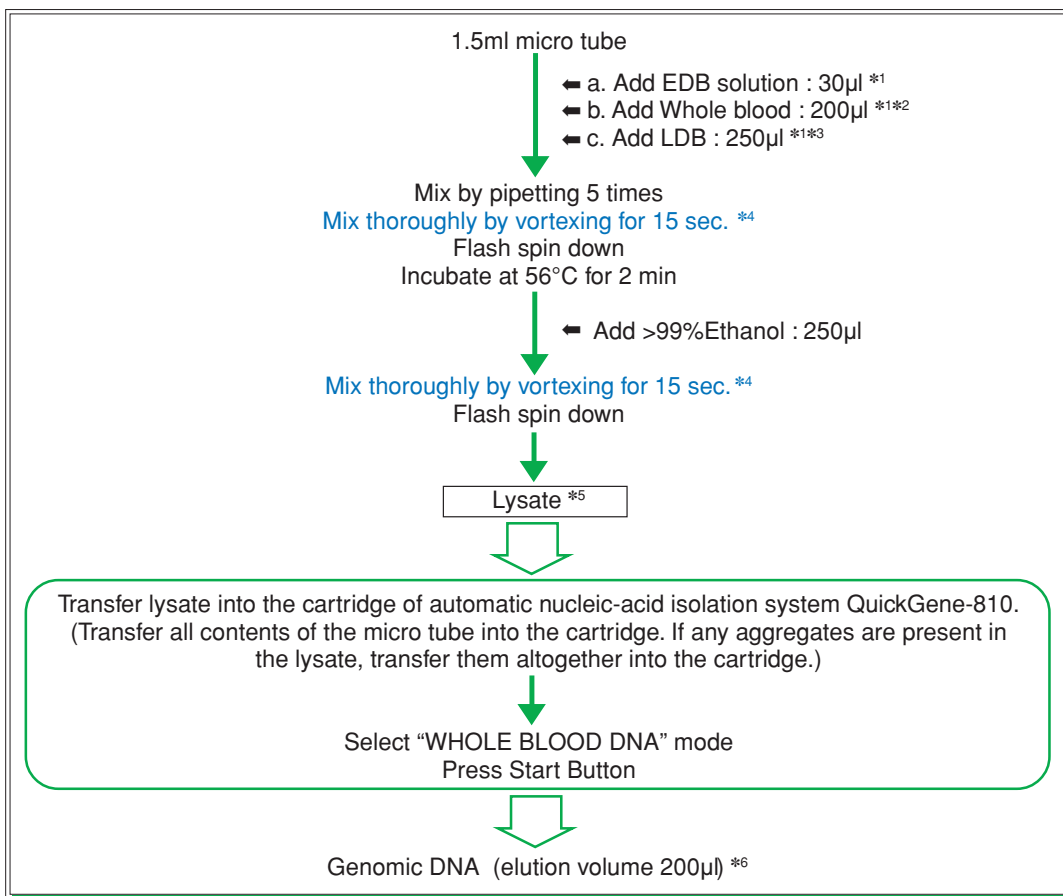
QuickGene DNA Whole Blood Kit S

Enables easy and rapid isolation of high purity genomic DNA from Human Whole Blood

Features

- Simultaneously extracts genomic DNA from 8 sets of Lysate in only 6 minutes
- Sophisticated genomic DNA isolation system without centrifugation
- Safety operation without using hazardous solvent such as phenol
- Isolated genomic DNA should be sufficient purity and yield for PCR, restriction enzyme digestion, Southern Blotting and other applications because of uncontaminated protein and chaotropic salt.

Protocol



*1 : Must follow the steps a, b, and c.

*2 : Recommend to use the whole blood collected in EDTA-2Na or EDTA-2K.

*3 : Proceed the step C immediately after adding whole blood.

*4 : Mix completely by vortexing at the maximum speed.

If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

*5 : Perform isolation within 30 min after lysate preparation.

*6 : This elution volume is initial value of "WHOLE BLOOD DNA" mode.

Components of the Kit

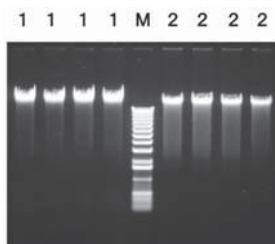
- Protease (EDB)
- Lysis buffer (LDB)
- Wash buffer (WDB)
- Collection buffer (CDB)
- Cartridges (CA)
- Collection tubes (CT)
- Caps (CAP)
- Waste tubes (WT)

Preparation of reagents

- Protease (EDB)
Add 3.3 ml of nuclease-free ultra pure water to the vial containing the freeze-dried protease, and dissolve it carefully.
- Wash buffer (WDB)
Add 160 ml of >99% ethanol into the bottle and mix with inversion the bottle gently before using.

Results : Genomic DNA isolation from Human Whole Blood

● AGE of isolated genomic DNA (Sample: 200µl of human whole blood)



M : 1k bp ladder
1 : Using QuickGene isolation system and reagents
2 : Comparison method using spin column

Genomic DNA was isolated from 200µl of human whole blood using QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S, and comparison method using spin column.

Isolation using QuickGene isolation system and reagents achieved a higher yield than the use of the comparison method using the spin column.

The isolated genomic DNA does not show protein, hemoglobin, or chaotropic salt contaminations. It is suitable for the next experiments such as PCR and restriction enzyme digestion directly.

● The yield of genomic DNA (Sample: 200µl of human whole blood)

| (µg) | Average | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---------|----------|----------|----------|----------|----------|
| QuickGene isolation system and reagents | 5.9 | 7.2 | 5.3 | 5.9 | 5.5 | 5.5 |
| Comparison method using spin column | 4.5 | 6.3 | 4.4 | 5.2 | 3.2 | 3.6 |

The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the high-yield isolation of genomic DNA from human whole blood.

● The purity of genomic DNA (determination of protein contamination) : $A_{260/280}$

| | Average | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---------|----------|----------|----------|----------|----------|
| QuickGene isolation system and reagents | 1.94 | 1.91 | 1.94 | 1.96 | 1.91 | 1.96 |
| Comparison method using spin column | 1.84 | 1.86 | 1.82 | 1.80 | 1.87 | 1.86 |

$A_{260/280}$: The ratio indicates the quality of nucleic acid from protein contamination ($A_{260/280} > 1.7$).
(Protein contamination decreases the ratio.)

The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of high-purity genomic DNA with little protein contamination.

● The purity of genomic DNA (determination of hemoglobin contamination) : A_{400}

| | Average | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---------|----------|----------|----------|----------|----------|
| QuickGene isolation system and reagents | 0.036 | 0.023 | 0.032 | 0.070 | 0.031 | 0.025 |
| Comparison method using spin column | 0.054 | 0.076 | 0.040 | 0.085 | 0.026 | 0.043 |

A_{400} : The absorbance at 400nm indicates hemoglobin contamination.

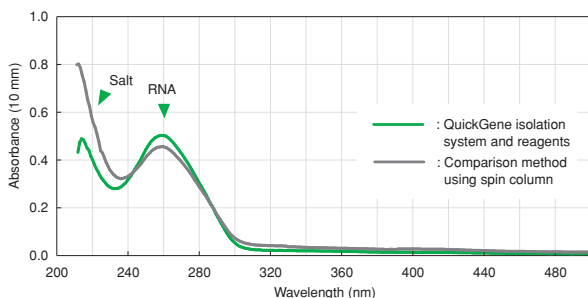
The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of high-purity genomic DNA from whole blood with little hemoglobin contamination.

● The purity of genomic DNA (determination of chaotropic salt contamination) : $A_{260/230}$

| | Average | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---------|----------|----------|----------|----------|----------|
| QuickGene isolation system and reagents | 1.61 | 1.76 | 1.69 | 1.43 | 1.76 | 1.42 |
| Comparison method using spin column | 1.12 | 1.21 | 0.89 | 1.07 | 1.24 | 1.21 |

$A_{260/230}$: The ratio indicates the quality of nucleic acid from chaotropic salt contamination.
(Chaotropic salt contamination decreases the ratio.)

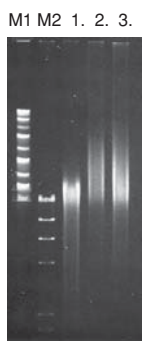
The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of high-purity genomic DNA with little contamination of chaotropic salt, which inhibits enzymatic reactions.



Contamination of chaotropic salt, which is protein denaturing agent, inhibits enzymatic reactions, and interferes with experiments performed by using isolated genomic DNA.

The presence of chaotropic salt in the isolated genomic DNA sample shows in increased absorbance at wavelengths of 240 nm or lower.

● Length of isolated genomic DNA



M1: MidRange PFG Marker II

M2: DNA/Hind III digest

1 : Comparison method using spin column (<~70kb)

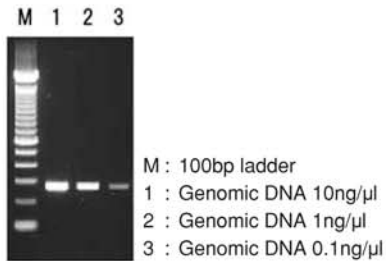
2 : Using QuickGene isolation system and reagents (<~140kb)

3 : Manual method using phenol/chloroform (<~140kb)

The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of long genomic DNA same as manual method using phenol/chloroform.

● PCR

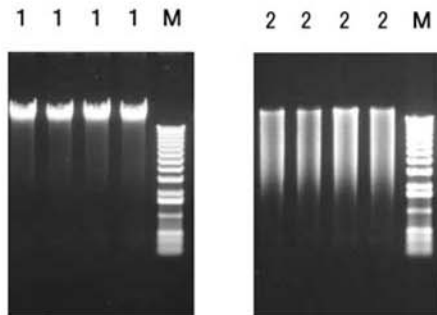
PCR performed on genomic DNA isolated from human whole blood using QuickGene-810



Serial dilution of isolated genomic DNA was used for PCR template to amplify p53 exon6 gene.
 PCR amplification was performed successfully by using 0.1ng/μl genomic DNA.

● Restriction Enzyme Digestion

AGE of EcoR I restriction enzyme digestion fragments of genomic DNA extracted from human whole blood using QuickGene isolation system and reagents



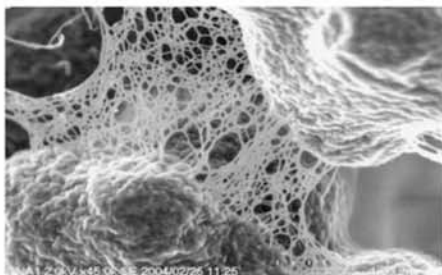
M : 1k bp ladder
 1 : Before digestion
 2 : After digestion using EcoR I

The eluted genomic DNA sample had been digested with EcoR I.

The success of enzyme digestion is shown by the comparison of lane1 and 2.

● SEM Image

Genomic DNA binding to patented Fuji Film's porous membrane



By using the patented Fuji Film's porous membrane, which made with advanced and independent technologies, QuickGene-810 quickly isolates genomic DNA from whole blood with high quality and high yield.