YTA Genomic DNA Extraction Mini Kit for whole Blood/ Cultured cell/ Plasma/Serum/Buffy coat/Body fluids/Lymphocytes,…

Cat No : YT9040 (For research Use only)

Introduction

Blood/Cultured cell Genomic DNA Extraction Mini Kit is an excellent tool offering a speedy and economic method to purify total DNA (e.g. genomic, mitochondrial and viral DNA) from whole blood (fresh or frozen), plasma, serum, buffy coat, body fluids, lymphocytes and cultured cells. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K, then binds DNA to silica-based membranes, washes DNA with ethanol-contained Wash Buffer or ddH2O. Compared with other harmful and time-consuming procedures, such as phenol/chloroform extraction and ethanol precipitation, this kit shortens the handling time within 1 hour. The size of purified DNA is up to 50 Kb (predominantly 20 – 30 Kb). After using Genomic DNA Extraction Mini Kit, the high quality total DNA can be used directly for the downstream applications.

Specification

**Sampling:** up to 200 µl of whole blood (with anti-coagulant), plasma, serum, buffy coat, or body fluids; up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS.

**Yield:** about 5 µg of total DNA from 200 µl of human whole blood; up to 50 µg of total DNA, depends on the sample types and the number of cells in the sample.

**Handling time:** within 1 hour, depends on the sample types.

Kit Contents

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG Buffer</td>
<td>15 ml</td>
</tr>
<tr>
<td>W1 Buffer*</td>
<td>22 ml</td>
</tr>
<tr>
<td>Wash Buffer**</td>
<td>10 ml</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>15 ml</td>
</tr>
<tr>
<td>Proteinase K*</td>
<td>11 mg</td>
</tr>
<tr>
<td>BG Mini Column</td>
<td>50 pcs</td>
</tr>
<tr>
<td>Collection Tube</td>
<td>100 pcs</td>
</tr>
<tr>
<td>Elution Tube</td>
<td>50 pcs</td>
</tr>
</tbody>
</table>

**Important Notes:**

1. Add 8 ml ethanol (96% - 100%) to W1 Buffer when first open.
2. Add 40 ml ethanol (96% - 100%) to Wash Buffer when first open.
3. Add 1.1 ml sterile ddH2O to each Proteinase K tube to make a 10mg/ml stock solution. Vortex and make sure Proteinase K has been completely dissolved. Store the stock solution at 4°C.
4. Buffer provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
5. Preheat a dry bath or a water bath to 60°C before the operation.

General Protocol:

Please Read Important Notes Before Starting The Following Steps.

HINTP: Preheat a 60°C dry bath or water bath for step 4.

1. Transfer up to 200 µl sample (whole blood, buffy coat) to a microcentrifuge tube (not provided). If the sample volume is less than 200 µl, add the appropriate volume of PBS.
2. (Optional): If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 minutes at room temperature.
3. Add 20 µl Proteinase K and 200 µl BG Buffer to the sample. Mix thoroughly by pulse-vortexing.
Do not add Proteinase K directly to BG Buffer.

4. Incubate at 60 °C for 15 minutes to lyse the sample. During incubation, vortex the sample every 3 – 5 minutes.

5. Briefly spin the tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96 - 100%) to the sample. Mix thoroughly by pulse-vortexing for 30 seconds.

7. Briefly spin the tube to remove drops from the inside of the lid.

8. Place a BG Column to a collection tube. Transfer the sample mixture (including any precipitate) carefully to BG Column. Centrifuge for 1 minute at 8000 rpm and discard the flow-through then place BG Column to a new Collection tube.

9. Immediately, Wash BG Column with 500 µl W1 Buffer by centrifuge for 1 minute at 14000 rpm then discard the flow-through.

10. Wash BG Column with 750 µl Wash Buffer by centrifuge for 1 minute at 14000 rpm then discard the flow-through.

11. Centrifuge at 14000 rpm for an additional 3 min to dry the column.

12. Place BG Column to Elution Tube.

13. Add 100 - 200 µl of Elution Buffer or ddH2O (pH 7.5 - 9.0) to the membrane center of BG Column. Stand BG Column for 3 min.

14. Centrifuge for 2 min at 14000 rpm to elute the DNA.

15. Store the DNA fragment at 4 °C or -20 °C.

Special Protocol

For cultured cells

1. Harvest Cells
   a. Cells grown in suspension
      i. Transfer the appropriate number of cell (up to 5 x 10⁶) to a 1.5 ml microcentrifuge tube (not provided)
      ii. Centrifuge at 300 x g for 5 minutes.
      iii. Remove the supernatant carefully and completely.
   b. Cells grown in monolayer
      i. Detach cells from the dish or flask by trypsinization or using a cell scraper.
      ii. Transfer the appropriate number of cell (up to 5 x 10⁶) to a 1.5 ml microcentrifuge tube (not provided).
      iii. Centrifuge at 300 x g for 5 minutes.
      iv. Remove the supernatant carefully and completely.

2. Resuspend cell pellet in PBS to a final volume of 200µl.

3. Follow the General Protocol starting from step 2.

Preparation of buffy coat

Centrifuge whole blood at 3,300xg for 10 minutes at room temperature and you will get three different fractions: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; the bottom layer contains concentrated erythrocytes. Process the General Protocol from Step 1 for buffy coat. Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.

Trouble Shooting
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Reasons/Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low or no yield of genomic DNA</td>
<td>1. Low amount of cells in the sample. Concentrate a larger volume of a new sample to 200 µl. If the sample is whole blood, prepare buffy coat (refer to Special Protocol on page 2).</td>
</tr>
</tbody>
</table>
|                                    | 2. Poor cell lysis  
|                                    | A. It is because of insufficient Proteinase K activity  
|                                    | . Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution.  
|                                    | B. It is because of insufficient mixing with BG Buffer  
|                                    | . Repeat the extraction procedure with a new sample. Mix the sample and BG Buffer immediately and thoroughly by pulse-vortexing.  
|                                    | C. It is because of insufficient incubation time.  
|                                    | . Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain. |
|                                    | 3. Ethanol is not added into the lysate before transferring sample mixture into BG Column.  
|                                    | . Repeat the extraction procedure with a new sample. |
|                                    | 4. Incorrect preparation of Wash Buffer  
|                                    | A. Ethanol is not added into Wash Buffer when first open.  
|                                    | . Make sure that the correct volumes of ethanol (96 ~ 100%) is added into Wash Buffer when first open. Repeat the extraction with a new sample.  
|                                    | B. The volume or the percentage of ethanol is not correct before adding into Wash Buffer.  
|                                    | . Make sure that the correct volumes of ethanol (96 ~ 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.  
|                                    | 5. Elution of genomic DNA is not efficient  
|                                    | A. pH of water (ddH₂O) for elution is acidic  
|                                    | . Make sure the pH of ddH₂O is between 7.5 ~ 9.0.  
|                                    | . Use Elution Buffer (provided) for elution.  
|                                    | B. Elution Buffer or ddH₂O is not completely absorbed by column membrane  
|                                    | . After Elution Buffer or ddH₂O is added, stand the BG Column for 5 minutes before centrifugation. |
| Brown residues remain on the column membrane after washing | 1. Poor Cell Lysis  
|                                    | A. It is because of insufficient Proteinase K activity  
|                                    | . Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution.  
|                                    | B. It is because of insufficient mixing with BG Buffer  
|                                    | . Repeat the extraction procedure with a new sample. Mix the sample and BG Buffer immediately and thoroughly by pulse-vortexing.  
|                                    | C. Poor cell lysis because of insufficient incubation time  
|                                    | . Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain. |
|                                    | 2. Ethanol is not added into the lysate before transferring sample mixture into BG Column.  
|                                    | . Repeat the extraction procedure with a new sample. |
|                                    | 3. Incorrect preparation of Wash Buffer  
|                                    | A. Ethanol is not added into Wash Buffer when first open.  
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|                                    | B. The volume or the percentage of ethanol is not correct before adding into Wash Buffer.  
|                                    | . Make sure that the correct volumes of ethanol (96 ~ 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.  
|                                    | 4. Column is clogged  
|                                    | 1. Blood sample contains clots  
|                                    | . Repeat the extraction procedure with a new sample. Mix the blood sample well with anticoagulant to prevent formation of blood clots.  
|                                    | 2. Sample is too viscous  
|                                    | . Reduce the sample volume. |
|                                    | 3. Insufficient activity of Proteinase K  
|                                    | . Use a fresh or well-stored Proteinase K Stock solution.  
|                                    | . Repeat the extraction procedure with a new sample. Do not add Proteinase K directly to BG Buffer. |
| Poor cells lysis                    | 1. Poor cell lysis because of insufficient Proteinase K activity  
|                                    | . Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution.  
|                                    | . Do not add Proteinase K directly to BG Buffer. |
|                                    | 2. Poor cell lysis because of insufficient mixing with BG Buffer  
|                                    | . Repeat the extraction procedure with a new sample. Mix the sample and BG Buffer immediately and thoroughly by pulse-vortexing. |
3. Poor cell lysis because of insufficient incubation time  
   - Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.

Ethanol is not added into the lysate before transferring sample mixture into BG Mini Column  
   - Repeat the extraction procedure with a new sample.

Incorrect preparation of Wash Buffer  

4. Ethanol is not added into Wash Buffer when first open  
   - Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Problem | Possible Reasons/Solution
---|---
A<sub>260</sub> / A<sub>280</sub> ratio of eluted DNA is low |  
5. The volume or the percentage of ethanol is not correct before adding into Wash Buffer  
   - Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

6. Genomic DNA is contaminated  
   - Do not wet the rim of the column during sample and buffer loading.

A<sub>260</sub> / A<sub>280</sub> ratio of eluted DNA is high |  
1. A lot of residual RNA in eluted DNA  
   - Follow the General Protocol step 2 to remove RNA.

2. BG Buffer added to the sample before adding RNase A  
   - Make sure that RNase A has been added to the sample before adding BG Buffer when using optional RNase step.

Degradation eluted DNA |  
1. Sample is old  
   - Always use fresh or well-stored sample for genomic DNA extraction.

2. Buffer for gel electrophoresis contaminated with DNase  
   - Use fresh running buffer for gel electrophoresis.