FastGene® miRNA Enhancer

FastGene® miRNA Enhancer is an additive solution for higher miRNA extraction.

Separately, one of the following kits is required depending on the purpose.

1. FastGene® RNA Basic kit
2. FastGene® RNA Basic kit + FastGene® RNA filter column single item
3. FastGene® RNA Premium kit

Be sure to read the FastGene® RNA Basic kit or FastGene® RNA Premium kit instruction manual in advance. Please check carefully before operating.

FG-RNAE-S (4 rxn)
FG-RNAE-25 (25 x 4 rxn)
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Component

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<th>Product name</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastGene® miRNA Enhancer (4 rxn)</td>
<td>FG-RNAE-S</td>
</tr>
<tr>
<td>FastGene® miRNA Enhancer (25 x 4 rxn)</td>
<td>FG-RNAE-25</td>
</tr>
</tbody>
</table>

Storage and Stability

Store FastGene® miRNA Enhancer at room temperature (15 ~ 25°C).

This reagent is solid or partially dissolved at room temperature (15 ~ 25°C). Before use, be sure to heat at 37°C for about 30 minutes to dissolve it completely.

Also, after thawing, it will solidify when left at room temperature, so keep warming until use.

Kit and protocol selection guide

Use this reagent with FastGene® RNA Basic kit and FastGene® RNA Premium kit

The protocol described here enables the recovery of small RNA (miRNA)

※ Please contact us, if you like to use a kit from different manufacturers.
## Quick guide: Extraction of total RNA including miRNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Standard protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample quantity</td>
<td>&lt; 1×10⁵ cultured cells</td>
</tr>
</tbody>
</table>
| Cell lysis and homogenisation | Add 350 µL buffer RL⁸¹
Thoroughly homogenization after addition |
| Clarification of lysate | ≥ 10,000 x g (RT: 20 ~ 25°C) 1 min
Transfer supernatant to a new collection tube |
| Optimize RNA binding condition (Addition of miRNA Enhancer) | Add 400 µL FastGene® miRNA enhancer solution
Mix by pipetting |
| RNA binding | FastGene® RNA binding column
Add up to 400 µL of sample solution
≥ 10,000 x g (RT: 20 ~ 25°C) 1 min
After discarding filtrate, return column to original tube (2.0 mL)
Repeat until all sample volume was applied |
| Membrane wash 1 | Add 400 µL buffer RW2⁸¹
≥ 10,000 x g (RT: 20 ~ 25°C) 30 s
Transfer the column to a new collection tube (2.0 mL) |
| Membrane wash 2 | Add 400 µL buffer RW2⁸¹
≥ 10,000 x g (RT: 20 ~ 25°C) 30 s
Transfer the column to a new collection tube (2.0 mL) |
| Membrane drying | Centrifugation at full speed (RT: 20 ~ 25°C) 1 min
Transfer the column to a new collection tube (1.5 mL) |
| Elution | Add 10-25 µL buffer RE
(Note: Add to the centre of the membrane)
≥ 10,000 x g (RT: 20 ~ 25°C) 1 min
After discarding FastGene® RNA binding column, collect eluate |

※1: These reagents must be prepared in advance.

Safety Stopping Point. After this step, storage of the sample at -70°C or lower is possible.
# Quick guide: Extraction of total RNA including miRNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Standard protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample quantity</strong></td>
<td>&lt; $1 \times 10^5$ cultured cells</td>
</tr>
<tr>
<td><strong>Cell lysis and homogenisation</strong></td>
<td>Add 350 µL buffer RL&lt;sup&gt;1&lt;/sup&gt; Thorough homogenization after addition</td>
</tr>
<tr>
<td><strong>Clarification of lysate</strong></td>
<td>Add lysate to FastGene&lt;sup&gt;®&lt;/sup&gt; RNA filter column [ ≥ 10,000 \times g (RT: 20 \sim 25°C) 1 \text{ min} ] Collect flow through after discarding FastGene&lt;sup&gt;®&lt;/sup&gt; RNA filter column</td>
</tr>
<tr>
<td><strong>Optimize RNA binding condition</strong></td>
<td>Add 400 µL FastGene&lt;sup&gt;®&lt;/sup&gt; miRNA enhancer solution Mix by pipetting</td>
</tr>
<tr>
<td><strong>RNA binding</strong></td>
<td>FastGene&lt;sup&gt;®&lt;/sup&gt; RNA binding column Add up to 700 µL of sample solution [ ≥ 10,000 \times g (RT: 20 \sim 25°C) 1 \text{ min} ] Discard flow through, return column to original tube (2.0 mL) Repeat until all sample volume was applied</td>
</tr>
<tr>
<td><strong>Membrane wash 1</strong></td>
<td>Add 400 µL buffer RW2&lt;sup&gt;1&lt;/sup&gt; [ ≥ 10,000 \times g (RT: 20 \sim 25°C) 30 \text{ s} ] Transfer column to a new collection tube (2.0 mL)</td>
</tr>
<tr>
<td><strong>Membrane wash 2</strong></td>
<td>Add 400 µL buffer RW2&lt;sup&gt;1&lt;/sup&gt; [ ≥ 10,000 \times g (RT: 20 \sim 25°C) 30 \text{ s} ] Transfer column to a new collection tube (2.0 mL)</td>
</tr>
<tr>
<td><strong>Membrane drying</strong></td>
<td>Centrifugation at full speed (RT: 20 \sim 25°C) 1 min Transfer column to a new collection tube (1.5 mL)</td>
</tr>
<tr>
<td><strong>Elution</strong></td>
<td>Add 10-25 µL buffer RE (\text{Note: Add to the centre of the membrane) [ ≥ 10,000 \times g (RT: 20 \sim 25°C) 1 \text{ min} ] After discarding FastGene&lt;sup&gt;®&lt;/sup&gt; RNA binding column, collect eluate</td>
</tr>
</tbody>
</table>

*<sup>1</sup>: These reagents require pre-preparation.

*<sup>3</sup>: Safety Stopping Point. After this operation, storage at -70°C or lower is also possible.


## Quick guide: Extraction of total RNA and miRNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Standard protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample quantity</td>
<td>&lt; $1 \times 10^5$ cultured cells</td>
</tr>
</tbody>
</table>
| Cell lysis and homogenisation             | Add 350 µL buffer RL<sup>1</sup>  
Thorough homogenization after addition  |
| Clarification of lysate                   | Add lysate to FastGene® RNA filter column  
$\geq 10,000 \times g$ (RT: 20 ~ 25°C) 1 min  
Collect flow through after discarding FastGene® RNA filter column |
| Optimize RNA binding condition            | Add 400 µL FastGene® miRNA enhancer solution  
Mix by pipetting  |
| RNA binding                              | FastGene® RNA binding column  
Add up to 700 µL of sample solution  
$\geq 10,000 \times g$ (RT: 20 ~ 25°C) 1 min  
Discard flow through, return column to original tube (2.0 mL)  |
| Membrane wash 1                           | Add 400 µL buffer RW2<sup>1</sup>  
$\geq 10,000 \times g$ (RT: 20 ~ 25°C) 30 s  
Transfer column to a new collection tube (2.0 mL)  |
| Membrane wash 2                           | Add 400 µL buffer RW2<sup>1</sup>  
$\geq 10,000 \times g$ (RT: 20 ~ 25°C) 30 s  
Transfer column to a new collection tube (2.0 mL)  |
| Membrane drying                           | Centrifugation at full speed (RT: 20 ~ 25°C) 1 min  
Transfer column to a new collection tube (1.5 mL)  |
| Elution                                   | Add 10-25 µL buffer RE  
(Note: Add to the centre of the membrane)  
$\geq 10,000 \times g$ (RT: 20 ~ 25°C) 1 min  
After discarding FastGene® RNA binding column, collect eluate  |

<sup>1</sup>: These reagents require pre-preparation.

<sup>S</sup>: Safety Stopping Point. After this operation, storage at -70°C or lower is also possible.
# FastGene® miRNA Enhancer

## Quick guide: miRNA extraction and isolation

<table>
<thead>
<tr>
<th>Step</th>
<th>Standard protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample quantity</td>
<td>&lt; 1×10^5 cultured cells</td>
</tr>
</tbody>
</table>
| Cell lysis and homogenisation             | Add 350 µL buffer RL<sup>1</sup>  
Thorough homogenization after addition   |
| Clarification of lysate                   | Add lysate to FastGene® RNA filter column  
≥ 10,000 x g (RT: 20 ~ 25°C) 1 min  
Collect flow through after discarding FastGene® RNA filter column |
| Optimize RNA binding condition            | Add 350 µL 70% Ethanol  
Mix by pipetting                                                                          |
| RNA binding of other RNA than miRNA      | FastGene® RNA binding column  
Add up to 700 µL of sample solution  
≥ 10,000 x g (RT: 20 ~ 25°C) 1 min  
Collect the flow through |
| Readjustment of conditions                | Add 300 µL FastGene® miRNA enhancer solution  
Mix by pipetting                                                                         |
| Column join miRNA                         | Add sample solution to FastGene® RNA mini-elute column  
≥ 10,000 x g (RT: 20 ~ 25°C) 1 min  
Discard flow through, return column to original tube (2.0 mL) |
| Membrane wash 1                           | Add 400 µL buffer RW2<sup>1</sup>  
≥ 10,000 x g (RT: 20 ~ 25°C) 30 s  
Transfer column to a new collection tube (2.0 mL) |
| Membrane wash 2                           | Add 400 µL buffer RW2<sup>1</sup>  
≥ 10,000 x g (RT: 20 ~ 25°C) 30 s  
Transfer column to a new collection tube (2.0 mL) |
| Membrane drying                           | Centrifugation at full speed (RT: 20 ~ 25°C) 1 min  
Transfer column to a new collection tube (1.5 mL) |
| Elution                                   | Add 10-25 µL buffer RE  
(Note: Add to the centre of the membrane)  
10,000 x g (RT: 20 ~ 25°C) 1 min  
After discarding FastGene® RNA mini-elute column, collect eluate |

<sup>1</sup>: These reagents require pre-preparation.

<sup>3</sup>: Safety Stopping Point. After this operation, storage at -70°C or lower is also possible.
**FastGene® miRNA Enhancer**

**Total RNA isolation including miRNA**

Before using the following procedure, incubate FastGene® miRNA Enhancer at 37°C for about 30 minutes to completely redissolve it. Also, check the FastGene® RNA Basic kit instruction manual and perform “Preliminary reagent preparation”. If you wish to use DNase I treatment, please check the support protocol of FastGene® RNA Basic kit.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Follow step 2 of the FastGene® RNA Basic kit protocol.</td>
</tr>
<tr>
<td>2</td>
<td>Centrifuge at ≥10,000xg for 1 min at RT (20 ℃ to 25 ℃). Transfer the supernatant to a new collection tube (1.5 mL).</td>
</tr>
<tr>
<td>3</td>
<td>Add 400 µL FastGene® miRNA enhancer solution to the clarified lysate and mix well by pipetting.</td>
</tr>
<tr>
<td>4</td>
<td>Load up to 700 µL of sample solution at a time onto the FastGene® RNA binding column and centrifuge at ≥10,000xg for 1 min at RT (20 ℃ to 25 ℃).</td>
</tr>
<tr>
<td>5</td>
<td>Discard the flow through from the collection tube (2.0 mL), and return the FastGene® RNA binding column to the original collection tube (2.0 mL). Repeat steps 5 - 6 until the whole sample volume is applied.</td>
</tr>
<tr>
<td>6</td>
<td>Add 400 µL of wash buffer RW2* to the FastGene® RNA binding column and centrifuge at ≥10,000xg for 30 seconds at RT (20 ℃ to 25 ℃). Transfer the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL). *Add ethanol in advance.</td>
</tr>
<tr>
<td>7</td>
<td>Add 400 µL of wash buffer RW2* to the FastGene® RNA binding column and centrifuge at ≥10,000xg for 30 seconds at RT (20 ℃ to 25 ℃). Transfer the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL). *Add ethanol in advance.</td>
</tr>
<tr>
<td>8</td>
<td>Centrifuge at full speed for 1 minute at RT (20 ℃ to 25 ℃) to dry the membrane. Transfer the FastGene® RNA binding column to a new collection tube (1.5 mL) and discard the flow through and the original collection tube (2.0 mL).</td>
</tr>
<tr>
<td>9</td>
<td>Add 10-25 µL of Elution Buffer RE to the centre of the FastGene® RNA binding column membrane.</td>
</tr>
<tr>
<td>10</td>
<td>Elute the purified RNA by centrifugation at ≥10,000xg for 1 minute at RT (20 ℃ to 25 ℃). Discard the FastGene® RNA binding column and collect the eluate. The RNA eluate in this step can be stored at -70°C or lower for over 1 year.</td>
</tr>
</tbody>
</table>

*Please be sure to read the precautions and important points before using FastGene® RNA Basic kit.*
### Total RNA extraction including miRNA

Before using the following procedure, incubate FastGene® miRNA Enhancer at 37°C for about 30 minutes to completely redissolve it. Also, check the FastGene® RNA Basic kit instruction manual and perform “Preliminary reagent preparation”. If you wish to use DNase I treatment, please check the support protocol of FastGene® RNA Basic kit.

#### Sample preparation - cell lysis and homogenization

1. Follow step 2 of the FastGene® RNA Basic kit protocol.

#### Lysate clarification

2. To reduce lysate viscosity and clarify, load sample onto FastGene® RNA filter column and centrifuge at \( \geq 10,000xg \) for 1 min at RT (20 ~ 25°C). Discard FastGene®RNA filter column and collect the flow through.

#### Adjustment of column binding conditions (addition of miRNA Enhancer)

3. Add 400 µL FastGene® miRNA enhancer solution to the clarified lysate and mix well by pipetting.

#### Column join

4. Load up to 700 µL of sample solution at a time onto the FastGene® RNA binding column and centrifuge at \( \geq 10,000xg \) for 1 min at RT (20 ~ 25°C).
5. Discard the flow through from the collection tube (2.0 mL), and return the FastGene® RNA binding column to the original collection tube (2.0 mL).
   Repeat steps 5 - 6 until the whole sample volume is applied.

#### Membrane wash 1

6. Add 400 µL of wash buffer RW2 to the FastGene® RNA binding column and centrifuge at \( \geq 10,000xg \) for 30 seconds at RT (20 ~ 25°C).
   Transfer the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL).
   *Add ethanol in advance.

#### Membrane wash 2

7. Add 400 µL of wash buffer RW2 to the FastGene® RNA binding column and centrifuge at \( \geq 10,000xg \) for 30 seconds at RT (20 ~ 25°C).
   Transfer the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL).
   *Add ethanol in advance.

#### Membrane drying

8. Centrifuge at full speed for 1 minute at RT (20 ~ 25°C) to dry the membrane.
   Transfer the FastGene® RNA binding column to a new collection tube (1.5 mL) and discard the flow through and the original collection tube (2.0 mL).

#### Elution

10. Elute the purified RNA by centrifugation at \( \geq 10,000xg \) for 1 minute at RT (20 ~ 25°C).
    Discard the FastGene® RNA binding column and collect the eluate.
    The RNA eluate in this step can be stored at -70°C or lower for over 1 year.

Please be sure to read the precautions and important points before using FastGene® RNA Basic kit.
# Total RNA extraction including miRNA

Before using the following procedure, incubate FastGene® miRNA Enhancer at 37°C for about 30 minutes to completely redissolve it. Also, check the FastGene® RNA Premium kit instruction manual and perform “Preliminary reagent preparation”. If you wish to use DNase I treatment, please check the support protocol of FastGene® RNA Premium kit.

## Sample preparation- cell lysis and homogenization

*Follow step 3 of the FastGene® RNA Premium kit protocol.

## Lysate clarification

1.  To reduce lysate viscosity and clarify, load sample onto FastGene® RNA filter column and centrifuge at \( \geq 10,000 \times g \) for 1 min at RT (20 ～ 25°C).  
   Discard FastGene® RNA filter column and collect the flow through.

## Adjustment of column binding conditions (addition of miRNA Enhancer)

2.  Add 400 µL FastGene® miRNA enhancer solution to the clarified lysate and mix well by pipetting.

## Column join

3.  Load up to 700 µL of sample solution at a time onto the FastGene® RNA binding column and centrifuge at \( \geq 10,000 \times g \) for 1 min at RT (20 ～ 25°C).  
4.  Discard the flow through from the collection tube (2.0 mL), and return the FastGene® RNA binding column to the original collection tube (2.0 mL).  
   Repeat steps 5 - 6 until the whole sample volume is applied.

## Membrane wash 1

5.  Add 400 µL of wash buffer RW2* to the FastGene® RNA binding column and centrifuge at \( \geq 10,000 \times g \) for 30 seconds at RT (20 ～ 25°C).  
   Transfer the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL).  
   *Add ethanol in advance.

## Membrane wash 2

6.  Add 400 µL of wash buffer RW2* to the FastGene® RNA binding column and centrifuge at \( \geq 10,000 \times g \) for 30 seconds at RT (20 ～ 25°C).  
   Move the FastGene® RNA binding column to a new collection tube (2.0 mL) and discard the flow through and the original collection tube (2.0 mL).  
   *Add ethanol in advance.

## Membrane drying

7.  Centrifuge at full speed for 1 minute at RT (20 ～ 25°C) to dry the membrane.  
   Transfer the FastGene® RNA binding column to a new collection tube (1.5 mL) and discard the flow through and the original collection tube (2.0 mL).

## Elution


9.  Elute the purified RNA by centrifugation at \( \geq 10,000 \times g \) for 1 minute at RT (20 ～ 25°C).  
   Discard the FastGene® RNA binding column and collect the eluate.  
   The RNA eluate in this step can be stored at -70°C or lower for over 1 year.

---

*Please be sure to read the precautions and important points before using FastGene® RNA Premium kit.*
miRNA isolation

Before using the following procedure, incubate FastGene® miRNA Enhancer at 37°C for about 30 minutes to completely redissolve it. Also, check the FastGene® RNA Premium kit instruction manual and perform “Preliminary reagent preparation”. If you wish to use DNase I treatment, please check the support protocol of FastGene® RNA Premium kit.

Sample preparation - cell lysis and homogenization

*Follow step 3 of the FastGene® RNA Premium kit protocol.

Lysate clarification

1. To reduce lysate viscosity and clarify, load sample onto FastGene® RNA filter column and centrifuge at ≥10,000xg for 1 min at RT (20 ~ 25°C). [Discard FastGene® RNA filter column and collect the flow through.]

Adjusting column binding conditions

2. Add 350 µL 70% ethanol to the clarified lysate and mix well by pipetting.

Column binding of other RNA than miRNA

3. Load up to 700 µL of sample solution at a time onto the FastGene® RNA binding column and centrifuge at ≥10,000xg for 1 min at RT (20 ~ 25°C). [Collect the filtrate containing miRNA that has passed through the column from the collection tube (2.0 mL) into another tube, and return the FastGene® RNA binding column to the original collection tube (2.0 mL). Repeat steps 5 - 6 until the whole sample volume is applied.]

Readjustment of column binding conditions (addition of miRNA Enhancer)

5. Add 300 µL of FastGene® enhancer solution to the filtrate containing miRNA collected in an extra tube. Mix thoroughly by pipetting.

miRNA column binding

6. Load all sample solution onto a FastGene® mini-elute column and centrifuge at ≥10,000xg for 1 min at RT (20 ~ 25°C).

7. Discard the flow through from the collection tube (2.0 mL) and return the FastGene® mini-elute column to the original collection tube (2.0 mL).

Membrane wash 1

8. Add 400 µL of wash buffer RW2* to the FastGene® RNA mini-elute column and centrifuge at ≥10,000xg for 30 seconds at RT (20 ~ 25°C). [Transfer the FastGene® RNA mini-elute column to a new collection tube (2.0 mL), discard the flow through and the original collection tube.]

*Add ethanol in advance.

Membrane wash 2

9. Add 400 µL of wash buffer RW2* to the FastGene® RNA mini-elute column and centrifuge at ≥10,000xg for 30 seconds at RT (20 ~ 25°C). [Transfer the FastGene® RNA mini-elute column to a new collection tube (2.0 mL), discard the flow through and the original collection tube.]

*Add ethanol in advance.

Please be sure to read the precautions and important points before using FastGene® RNA Premium kit.
Membrane drying

10. Centrifuge at RT (20 ~ 25°C) for 1 minute at full speed to dry the membrane.
    Transfer FastGene® RNA mini-elute column to a new collection tube (1.5 mL), discard the filtrate
    that has passed through the column and the original collection tube (2.0 mL).

Elution

12. Elute the purified RNA by centrifugation at ≥10,000xg for 1 minute at RT (20 ~ 25°C).
    Discard FastGene® RNA mini-elute column and collect the eluate.
    The RNA eluted in this step can be stored at −70°C or lower for over 1 year.

Please be sure to read the precautions and important points before using the FastGene® RNA Premium kit.

Order information

<table>
<thead>
<tr>
<th>Product name</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastGene® miRNA Enhancer Trial kit (4 rxn)</td>
<td>FG-RNAE-S</td>
</tr>
<tr>
<td>FastGene® miRNA Enhancer (25 x 4 rxn)</td>
<td>FG-RNAE-25</td>
</tr>
</tbody>
</table>

Related Products

<table>
<thead>
<tr>
<th>Product name</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastGene® RNA Basic Kit (6 preps)</td>
<td>FG-80006</td>
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<tr>
<td>FastGene® RNA Basic Kit (50 preps)</td>
<td>FG-80050</td>
</tr>
<tr>
<td>FastGene® RNA Basic Kit (250 preps)</td>
<td>FG-80250</td>
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<tr>
<td>FastGene® RNA filter column single item</td>
<td>FG-81FC050</td>
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<tr>
<td>FastGene® RNA Premium kit (6 preps)</td>
<td>FG-81006</td>
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<tr>
<td>FastGene®RNA Premium kit (50 preps)</td>
<td>FG-81050</td>
</tr>
<tr>
<td>FastGene® RNA Premium kit (250 preps)</td>
<td>FG-81250</td>
</tr>
</tbody>
</table>

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Contact us

For more product information, contact details, questions, troubleshooting, please give us a call,
write an email or visit our webpage.

Nippon Genetics Europe GmbH

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