PRODUCT INFORMATION

Product Name: DNA fragments for DynaMarker RNA High Probe
Code No.: DM173
Size: 5 μg (50 μl), 0.1 μg/μl

This product is research use only

Description:
DNA fragments for DynaMarker RNA High Probe is a product for detection of DynaMarker RNA High (DM160) and DynaMarker RNA High AGN (in DynaMarker RNA Easy Measurement N, DM170) on hybridization. The DNA fragments for DynaMarker RNA High Probe consists of two DNA fragments, approximately 170 bp and 200 bp. The DNA fragments have phosphorylated 5' protruding ends. After labeling it by enzymatic end labeling methods or non-isotopic labeling methods, the DNA fragments for DynaMarker RNA High Probe is used for a DNA probe to detect all RNA bands of DynaMarker RNA High and DynaMarker RNA High AGN on hybridization.

Storage buffer:
10 mM Tris-HCl (pH 8.0) buffer

Storage condition:
Store at -20 °C.
For short term storage (< several days), storage at 4 °C is permissible.

Quality Control (Test of RNase Activity):
After 12-16 hr incubation of 0.1 μg of DNA fragments for DynaMarker RNA High Probe with 50 ng of RNA at 37 °C, no visible degradation of the RNA is observed in gel electrophoresis.
PRODUCT INFORMATION

Additional Information

Procedure of Northern transfer with DynaMarker RNA Easy Measurement N (DM170)

Note:
- RNA is very sensitive to degradation by nuclease. To prevent nuclease contamination, extreme care is required for manipulation. Wear gloves and use clean apparatus. Glassware should be pretreated with diethyl pyrocarbonate (DEPC). Nuclease-free disposable plasticware should be used. Solutions and reagents to mix RNA should be high grade and nuclease-free.
- Formamide is suspected to be harmful. It is irritate to the eyes and skin.
- Ethidium bromide (contained in RNA loading buffer AG+) is a strong mutagen and suspected to be toxic.
- Formaldehyde is carcinogen and toxic. Avoid breathing the vapors.
- Wear appropriate gloves and safety glasses in using solutions and materials containing formamide, ethidium bromide and/or Formaldehyde. Gel containing formaldehyde should be prepared and used in a chemical hood.
- For electrophoresis procedure, see detail Product Information of DynaMarker RNA Easy Measurement N (DM170)

I. Electrophoresis of DynaMarker RNA HighAGN

Both agarose gels electrophorezed by non-denaturing gel method and by denaturing gel method can be subjected to northern transfer. In this protocol, the two procedure, non-denaturing agarose gel method (A) and denaturing agarose gel method (B) are described. See detail Product Information of DynaMarker RNA Easy Measurement N (DM170) for electrophoresis procedure.

A. Non-denaturing agarose gel method

A.1. Preparation of non-denaturing agarose gel

Add 1.3 g of agarose to 100 ml of 1 × TAE in a flask, dissolve the agarose in a microwave. After swirling to mix them, quickly pour the agarose into a gel mold and set a comb just as agarose gel for DNA electrophoresis. The preferable thickness of gel is approximately 3 - 7 mm for northern transfer.

A.2. Preparation of formaldehyde –added RNA loading buffer AG+

Formaldehyde –added RNA loading buffer AG+ is prepared as below.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA loading buffer AG+ (DM172)</td>
<td>95 μl</td>
</tr>
<tr>
<td>37 % formaldehyde solution</td>
<td>5 μl</td>
</tr>
<tr>
<td>Formaldehyde –added RNA loading buffer AG+</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

*After mixing with formaldehyde, the solution is not stable, do not use it more than 6 hours after preparation.

Formaldehyde is supplied as a 37-40 % W/V (12.3 M) solution that contain a stabilizer such as methanol (10 - 15 %). The 37 % formaldehyde solution is used for mixing with RNA loading buffer AG+ and also denaturing agarose gel containing formaldehyde. For instance, Sigma-Aldrich supplies formaldehyde solution, 36.5 - 38 % in water, for molecular biology, which contains 10 -15 % methanol.
A.3. Denaturation of RNA

Prepare denaturated $\text{DynaMarker\ RNA\ High\ AGN (DM171)}$ in a small tube as below.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{DynaMarker\ RNA\ High\ AGN}$</td>
<td>0.5-2 $\mu$l $^*$</td>
</tr>
<tr>
<td>formaldehyde–added RNA loading buffer AG$^+$</td>
<td>3 $\mu$l $^{**}$</td>
</tr>
<tr>
<td>ddH$_2$O</td>
<td>to 5 $\mu$l</td>
</tr>
</tbody>
</table>

After mixing, heat the RNA solution at 75 $^\circ$C for 3 min, then quickly transfer the tube on ice.

Mixture of formaldehyde-added RNA loading buffer AG$^+$ and RNA is not stable, use it promptly.

$^*$ Required $\text{DynaMarker\ RNA\ High\ AGN}$ amount depends on experiments. For northern detection of $\text{DynaMarker\ RNA\ High\ or\ DynaMarker\ RNA\ High\ AGN}$, 0.5 $\mu$l of the size marker is fully enough. More than 0.05 $\mu$g of RNA band can be detected on gel under UV light with treatment of formaldehyde-added RNA loading buffer AG$^+$.

$^{**}$ Use freshly prepared formaldehyde-added RNA loading buffer AG$^+$.

$^{***}$ Use more than one volume of RNA solution.

A.4. Loading and electrophoresis

Set up the prepared agarose gel in a horizontal electrophoresis apparatus submerged in 1 × TAE. Load the above denatured RNA solution to a well and start electrophoresis as just DNA electrophoresis. After the tracking dye has migrated an appropriate distance from wells, stop the electrophoresis. RNA bands can be seen under UV illumination. You can take photographs of gel for reference.

B. Denaturing agarose gel method

B.1. Preparation of denaturing agarose gel

Add 1 g of agarose to 85 ml of H$_2$O in a flask, dissolve the agarose in a microwave. Add 10 ml of 10 × MOPS buffer to the agarose solution, then allow it in a flask to cool to 55 $^\circ$C. Add 5.4 ml of 37 % formaldehyde solution to the agarose solution, mix them, quickly pour the agarose into a gel mold and set a comb in a fume hood. Cover the gel with 1 × MOPS buffer until use. Formaldehyde is supplied as a 37-40 % W/V (12.3 M) solution that contain a stabilizer such as methanol (10-15 %). The 37 % formaldehyde solution is used for mixing with RNA loading buffer AG$^+$. The preferable thickness of gel is approximately 3 - 7 mm for northern transfer.

B.2. Formaldehyde-added RNA loading buffer AG$^+$ is prepared as below.

This procedure is the same as that of A.2. Preparation of formaldehyde–added RNA loading buffer AG$^+$.

B.3. Denaturation of RNA

This procedure is the same as that of A.3. Denaturation of RNA.
B. 4. Loading and electrophoresis
Set up the prepared agarose gel containing formaldehyde in a horizontal electrophoresis apparatus submerged in 1 \times \text{MOPS buffer}. Load the denatured RNA solution to a well and start electrophoresis. After the tracking dye has migrated an appropriate distance from wells, stop the electrophoresis. RNA bands can be seen under UV illumination. You can take photographs of gel for reference.

II. Northern transfer of DynaMarker RNA HighAGN onto nylon membrane
1. Northern transfer method
There are several protocols for northern transfer. In this protocol, a widespread capillary method (upward) is described. Alternatively downward transfer method or electroblotting method may be used.

2. Treatment of electrophoresised agarose gel
After electrophoresis, trim away any unused area of the agarose gel (for example, upper area of wells). Soak the gel with RNase-free water for 15 min, two times, with a gentle agitation. It is not necessary to fragment the RNA or neutralized the agarose gel, which are required for southern transfer.

3. Preparation of membrane and filter paper
Use gloves and forceps to handle the nylon membrane. Cut a piece of nylon membrane about 1 mm larger than the gel in both dimension. Cut Whatman 3MM filter paper and towel paper to the same dimensions of the gel. Cut another Whatman 3MM filter paper enough to cover the area of the gel for a bridges between transfer buffer reservoir and filter paper under the gel.

4. Transfer (See Fig. 2)
1) Assemble the transfer apparatus as Fig. 2. Fill the glass dish with enough 20 \times \text{SSC}^*.
2) Wet a bridge of filter paper with 20 \times \text{SSC}, place it on a solid support in the glass dish.
3) Wet 2-3 pieces of gel-size filter paper with 20 \times \text{SSC}, place it on the bridge of filter paper.
4) Place the gel on the filter paper. Try to avoid getting air bubbles between the gel and filter paper.
5) Float the nylon membrane on the surface of distilled water and submerge in the water to be wet completely.
6) Surround the sides of gel with strips of Parafilm to prevent the two layers of filter paper from coming in contact with each other.
7) Place the wet nylon membrane on the top of the gel. Make sure that there are no air bubbles between the gel and nylon membrane.
8) Wet 2-3 pieces of gel-size filter paper with 20 \times \text{SSC}, place it on the nylon membrane
9) Put gel-size paper towels on top of the filter paper to a height of 5-8 cm, and add a weight (about 500 g) to hold everything in place.
10) Allow transfer requires approximately 2-3 hrs but may be carried out overnight. As the paper towels become wet, they should be replaced.

11) After transfer remove paper towels and filter paper. For orientation purposes, cut a small notch in the upper right hand corner of the membrane and remove membrane from gel.

12) Wash membrane in 5 × SSC for 5 min after transfer.

13) Bake membrane at 65 - 80 °C for 30 minutes - 1 hr or until completely dry (A vacuum oven is not necessary when baking nylon membrane).
   • Alternatively, exposure the nylon membrane to a UV source (254 nm). Total exposure should be approximately 1.6 KJ/m2 for wet membranes and 160 J/m2 for dry membrane.

14) Store membrane desiccated at 4 °C until hybridization, the blot is stable at 4 °C for several months.

20 × SSC*: 3.0 M Sodium Chloride, 0.3 M Sodium Citrate, pH 7.0

III. Reference: