microRNA Marker

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

N2102S 009110613062

N2102S

Marker: 100 gel lanes (6 µg)
12 ng/µl
Lot: 0091106
Store at –20°C
Exp: 6/13

Probe (21-mer): 10 µg
20 ng/µl
Lot: 0091106
Store at –20°C
Exp: 6/13

Description: The microRNA Marker is a set of three synthetic single-stranded RNA oligonucleotides 17, 21 and 25 residues long that have free 5´ ends (i.e., no 5´ phosphate groups). These oligonucleotides can be used as size markers on denaturing polyacrylamide gels and Northern blots. The microRNA Marker is best visualized by staining with SYBR-Gold instead of ethidium bromide (Figure 1).

The three single-stranded RNA oligonucleotides contain the same core sequence so they can be detected by hybridization with the same probe. The sequences of the RNA oligonucleotides are as follows:

25-mer: 5´AGAG**CAGUGGCUGGUUGAGAU**UUAA 3´
21-mer: 5´AG**CAGUGGCUGGUUGAGAU**UU 3´
17-mer: 5´**CAGUGGCUGGUUGAGAU** 3´

Note: The sequence in bold is common to all three oligos.

A synthetic single-stranded 21-mer DNA oligonucleotide probe complementary to the above sequences is included. This oligonucleotide probe is biotinylated at the 3´ end and has a free 5´ end so it can also be labeled with γ-^{32}P-ATP and T4 Polynucleotide Kinase (NEB# M0201). The sequence of the oligonucleotide probe is as follows:

5´AAATCTCAACCAGCCACTGCT 3´-Biotin

Supplied in: The microRNA Marker is provided in a ready-to-load solution containing 4 M urea and 0.04% Orange G. The microRNA Marker probe is resuspended in water.

Usage Notes: The microRNA Marker is provided in a ready-to-load denaturing solution. Denature by heating for 3–5 minutes at 95°C and place on ice. Load 5–10 µl for staining with SYBR Gold in denaturing gels. In Northern blots, less than 1 µl (12 ng) is sufficient for detection by hybridization.

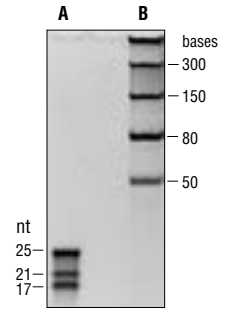

Figure 1: *The microRNA Marker (A) and the Low Range ssRNA Marker (B, NEB #N0364). 5 µl (60 ng) of the microRNA Marker and 4 µl (0.2 µg) of the Low Range ssRNA Ladder are loaded on a 15% polyacrylamide-urea gel and stained with SYBR Gold (Molecular Probes) for 1 minute and photographed on a transilluminator.*

The Orange G loading buffer migrates faster than the smallest band, and migrates approximately as far as the nucleotides.

Oligonucleotide Probe Usage:

The provided biotinylated probe DNA oligonucleotide can be used directly in hybridization and detected with the Phototope Star Detection Kit (NEB #N7020). Use 1 to 10 µl of Probe (20-200 ng) per 10 ml hybridization solution. Hybridize and wash the blot at 35–40°C depending on the hybridization solution used. More details can be found in reference 1 and the Phototope Star Detection Kit manual (2).

Alternatively, the probe can be labeled with T4 Polynucleotide Kinase (NEB #M0201) and radioactive γ-^{32}P-ATP using the following protocol:

1. Mix the following components in a sterile microfuge tube:

Oligonucleotide Probe	1–5 µl
10X T4 Polynucleotide Kinase Reaction Buffer	2.0 µl
γ-^{32}P-ATP (5 µCi/µl)	1–2 µl
T4 Polynuclotide Kinase	1 µl
Sterile dH_2O	X µl
Total volume	20 µl

(See other side)

CERTIFICATE OF ANALYSIS

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The Orange G loading buffer migrates faster than the smallest band, and migrates approximately as far as the nucleotides.
Figure 1: The microRNA Marker (A) and the Low Range ssRNA Marker (B, NEB #N0364). 5 µl (60 ng) of the microRNA Marker and 4 µl (0.2 µg) of the Low Range ssRNA Ladder are loaded on a 15% polyacrylamide-urea gel and stained with SYBR Gold (Molecular Probes) for 1 minute and photographed on a transilluminator.
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1. Mix the following components in a sterile microfuge tube:
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10X T4 Polynucleotide Kinase Reaction Buffer 2.0 µl
γ-32P-ATP (5 µCi/µl) 1–2 µl
T4 Polynuclotide Kinase 1 µl
Sterile dH2O X µl
Total volume 20 µl
(See other side)
CERTIFICATE OF ANALYSIS

2. Incubate for 30 minutes at 37°C.
3. Purify labeled probe using a G-25 spin column.

References:

1. Sambrook, J. and Russel, D.W. (2001) *Molecular Cloning: A Laboratory Manual,* (3rd ed.) pp. 7.1–7.56 Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. http://www.neb.com/nebecomm/products/productN7020.asp

Companion Products:

Phototope® Star Detection Kit	
#N7020S	20,000 cm^2
siRNA Marker	
#N2101S	4.5 µg
Low Range ssRNA Marker	
#N0364S	25 µg
T4 Polynucleotide Kinase	
#M0201S	500 units
#M0201L	2,500 units

2. Incubate for 30 minutes at 37°C.
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References:

1. Sambrook, J. and Russel, D.W. (2001) *Molecular Cloning: A Laboratory Manual,* (3rd ed.) pp. 7.1–7.56 Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
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Companion Products:

Phototope® Star Detection Kit	
#N7020S	20,000 cm^2
siRNA Marker	
#N2101S	4.5 µg
Low Range ssRNA Marker	
#N0364S	25 µg
T4 Polynucleotide Kinase	
#M0201S	500 units
#M0201L	2,500 units

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