

microRNA Marker



1-800-632-7799
info@neb.com
www.neb.com



N1202S 012150317031

N2102S

Marker: 100 gel lanes (6 µg)
12 ng/µl
Lot: 0121503
Store at -20°C
Exp: 3/17

Probe (21-mer): 10 µg
20 ng/µl
Lot: 0121503
Store at -20°C
Exp: 3/17

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**CAGUGGCGUGGU**GAGAUUUA 3'
21-mer: 5'AG**CAGUGGCGUGGU**GAGAUUU 3'
17-mer: 5'**CAGUGGCGUGGU**GAGAU 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 -³²P-ATP and T4 Polynucleotide Kinase (NEB# M0201). The sequence of the oligonucleotide probe is as follows:

5'AAATCTCAAC**CAGCCACTGCT** 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.

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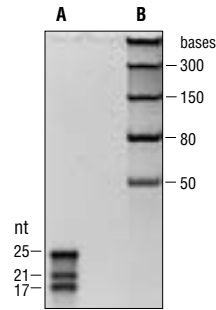


Figure 1: The microRNA Marker (A) and the Low Range ssRNA Marker (B, NEB #N0364). 5 µl (60 ng) of the microRNA Marker and 4 µl (0.2 µg) of the Low Range ssRNA Ladder are loaded on a 15% polyacrylamide-urea gel and stained with SYBR Gold (Molecular Probes) for 1 minute and photographed on a transilluminator.

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.

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 -³²P-ATP using the following protocol:

- Mix the following components in a sterile microfuge tube:

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

(See other side)

CERTIFICATE OF ANALYSIS

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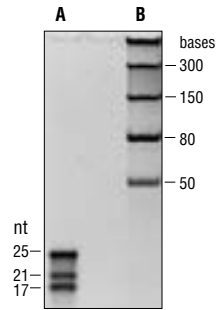


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T4 Polynucleotide Kinase	1 µl
Sterile dH ₂ O	X µl
Total volume	20 µl

(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

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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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