The microRNA Marker is a set of three synthetic single-stranded RNA oligonucleotides consisting of 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 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:

1. Mix the following components in a sterile microtube:
   - Oligonucleotide Probe 1–5 µl
   - 10X T4 Polynucleotide Kinase Kinase 2.0 µl
   - Reaction Buffer 1–2 µl
   - T4 Polynucleotide Kinase 1 µl
   - Sterile dH2O X µl
   - Total volume 20 µl

(See other side)

**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:

1. Mix the following components in a sterile microtube:
   - Oligonucleotide Probe 1–5 µl
   - 10X T4 Polynucleotide Kinase Kinase 2.0 µl
   - Reaction Buffer 1–2 µl
   - T4 Polynucleotide Kinase 1 µl
   - Sterile dH2O X µl
   - Total volume 20 µl

(See other side)
2. Incubate for 30 minutes at 37°C.

References:

Companion Products:
Phototope® Star Detection Kit
#N7020S 20,000 cm²
siRNA Marker
#N2101S 4.5 µg
Low Range ssRNA Marker
#N0364S 25 µg
T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units