p19 miRNA Detection Kit

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E3312S 001110813081

E3312S

50 rxns (600 µl vol) **Lot: 0011108**

Store at 4°C **Exp: 8/13**

Description: The p19 miRNA Detection Kit uses the high affinity binding properties of the p19 protein to detect miRNAs that form hybrids with a specific probe. The p19 protein (19 kDa) from Carnation Italian Ringspot Virus (CIRV) binds 21–23 mer dsRNAs with nanomolar affinity (1) in a size dependent and sequence independent manner. The p19 is expressed as an amino terminal fusion of the maltose binding protein (MBP) and a carboxy terminal fusion of the chitin binding domain (CBD). The MBP aids in p19 purification and the CBD allows p19 to bind very tightly to Chitin Magnetic Beads (NEB #E8036). The p19 fusion protein bound to chitin magnetic beads (p19 beads) has the same binding properties as the native p19; it selectively binds siRNAs that are 21–23 bases long but does not bind ssRNA or dsDNA of the same length (2,3). After siRNA or hybrids of miRNA:RNA-probe are bound to the p19 beads they can easily be isolated in a small volume using a Magnetic Separation Rack (NEB #S1506 or #S1509) and eluted with SDS. The p19 beads can also enrich siRNAs greater than 3,000 fold from a mixture of total cytoplasmic RNA.

The use of p19 for miRNA detection has the dual advantage of high affinity and size dependent binding of dsRNA. Hybridization of a labeled RNA probe complementary to a specific miRNA creates a dsRNA hybrid that selectively binds to p19 chitin magnetic beads. The unbound probe is removed by washing. The eluted double stranded miRNA:RNA-probe can then be counted or analyzed on an polyacrylamide gel. Using a ^{32}P radioactive RNA probe, less than 10 picograms of miRNA can be detected in a million fold excess of unlabeled RNA (Figure 1)(3).

Source: The p19 siRNA Binding Protein gene is from the Carnation Italian Ringsport virus (CIRV). The p19 protein is expressed in *E. coli* and purified as MBP-p19-CBD fusion protein.

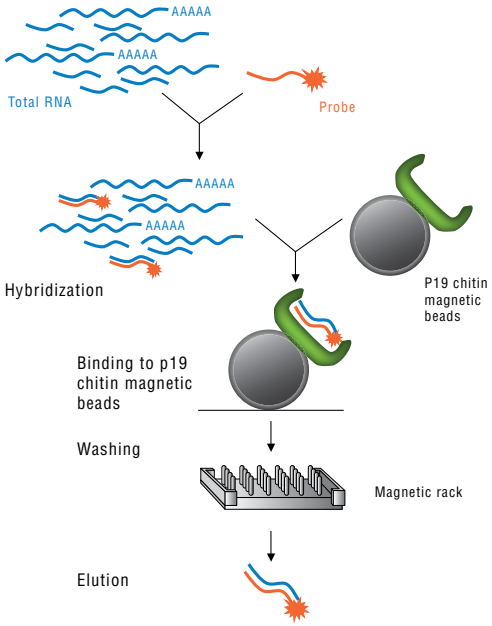


Figure 1: *The miRNA-probe, shown in red, is hybridized with a total RNA extract and bound to the chitin magnetic beads. The p19 protein, in green, is linked to the beads via the chitin binding domain. The unbound probe is removed and the miRNA:RNA-probe can be eluted and quantitatively measured.*

Advantages:

- Rapid detection of miRNA requiring few steps
- Sensitivity in the low picogram range with very low background
- Quantitative detection that is linear over three logs
- The p19 chitin magnetic beads are stable for months at 4°C
- Size dependent sequence independent binding of miRNA:RNA-probe hybrids

Applications:

- miRNA detection without doing a Northern blot
- Affinity purification of siRNA

Kit Components:

p19 siRNA Binding Protein (10 units/µl). Store at –20°C.	200 µl
BSA Treated Chitin Magnetic Beads supplied in 1X p19 Binding Buffer with 10X BSA (1 mg/ml). Store at 4°C.	600 µl
10X p19 Binding Buffer Store at –20°C.	300 µl
100X BSA (10 mg/ml) Store at –20°C.	1.5 ml
RNase Inhibitor, Murine (40,000 units/ml) Store at –20°C	75 µl
25X p19 Wash Buffer Store at 4°C.	10 ml
1X p19 Elution Buffer Store at 4°C.	1.5 ml

1X p19 Binding Buffer:
20 mM Tris-HCl
100 mM NaCl
1 mM EDTA
1 mM TCEP
0.02% Tween-20
(pH 7.0 @ 25°C)

1X p19 Wash Buffer:
20 mM Tris-HCl
100 mM NaCl
1 mM EDTA
(pH 7.0 at 25°C)
supplement with 1X BSA (100 µg/ml)

1X p19 Elution Buffer:
20 mM Tris-HCl
100 mM NaCl
1 mM EDTA
0.5 % SDS
(pH 7.0 at 25°C)

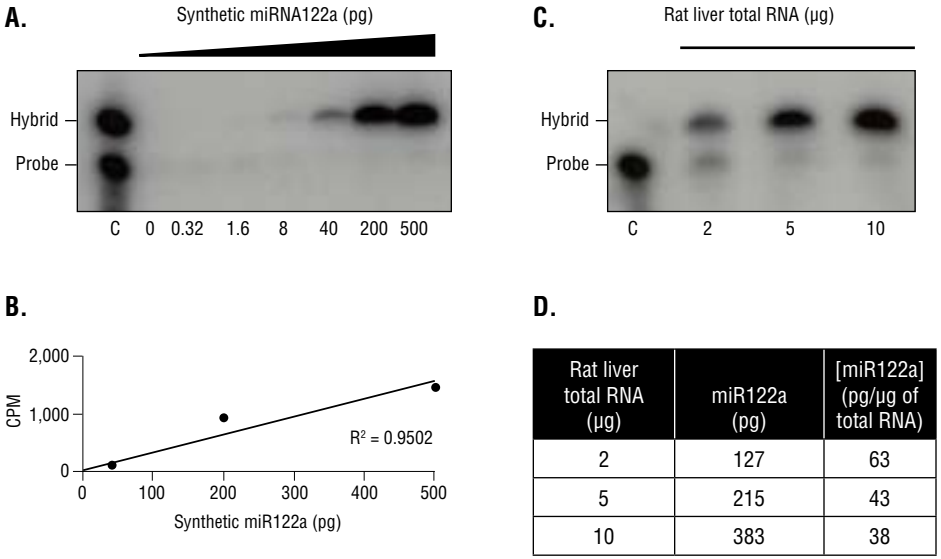
Notes:
miRNA Probe Design: Most miRNAs are 19 to 25 bases in length. To detect miRNA using p19 beads, the optimum size RNA probe is 19 nucleotides long and complementary to a target miRNA. The probe should form a blunt ended duplex with one end of the miRNA, however, the miRNA can extend several nucleotides past the other end of the RNA probe and still bind tightly to the p19 beads. The RNA probe should not have a 5´ phosphate so it can be labeled (see following section).

Protocol for miRNA Detection:

1. Labeling of miRNA Probe: Combine the following in a sterile microcentrifuge tube:
   - 20–100 ng 5´ OH RNA oligo
   - 2 µl T4 Polynucleotide Kinase Buffer (10X)
   - 20–100 µCi of γ-^{32}P-ATP with 6,000 Ci/mM
   - 2 µl T4 Polynucleotide Kinase (10 units/µl) (NEB #M0201)
   - Add H_2O to 20 µl
2. Incubate at 37°C for 60 minutes followed by 20 minutes at 65°C to inactivate the enzyme. Use CentriSep column (Princeton Separation) to remove the unincorporated isotope and measure the specific activity using a scintillation counter.
3. Standard Curve: For quantitative detection of miRNAs, a standard curve is made using decreasing amounts of a synthetic miRNA (from 300 to 1 pg) and a constant amount of the ^{32}P labeled probe (~500 pgm). The hybridization can contain several micrograms of total RNA, like yeast or bacteria, which does not contain the miRNA of interest. This will mimic miRNA detection in the unknown samples. The counts bound and eluted from the p19 beads can be plotted against the pg of the miRNA in the reaction. The steps for hybridization and processing the beads are described below (See Figure 2).
4. Hybridization: Typically, 1–0.5 ng of ^{32}P-probe was hybridized to a total RNA sample (from 1–10 µg) by incubating at 55°C–65°C (depends on the Tm of your probe) for 2 hours in 10 µl containing 1X

(See other side)

CERTIFICATE OF ANALYSIS



Rat liver total RNA (µg)	miR122a (pg)	[miR122a] (pg/µg of total RNA)
2	127	63
5	215	43
10	383	38

Figure 2: Standard curve for miR-122a in rat liver RNA. *(A) An autoradiograph of a 20% polyacrylamide TBE gel was used for the miR-122a standard curve. Increasing amounts of synthetic miR-122a were hybridized to a constant amount of ^{32}P labeled probe in the presence of a large excess of Jurkat cell RNA. The miR-122a:RNA probe hybrid was bound to the p19 beads, washed and eluted as described. The lane labeled C is the size standard for miR-122a:probe hybrid and probe. (B) Standard curve for miR-122a detection was based on an aliquot eluted from beads used for the gel in panel A. (C) Detection of miR-122a in different amounts of total rat liver RNA. Eluted miR-122a:probe hybrids were analyzed as described in panel A. The size standard for ssRNA probe is in lane C. (D) Results of quantitative measurement of miR-122a in total rat liver RNA. Three different amounts of total RNA were used. The standard curve in panel B was used to convert cpm to pg of miR-122a.*

p19 Binding Buffer. Temperature depends upon base composition of the RNA. Hybridization allows the probe to form duplex with the endogenous target miRNA. We recommend the use of a PCR machine to avoid evaporation.

5. Binding of p19 to BSA Treated Chitin Magnetic Beads: Resuspend the BSA treated chitin magnetic beads with a brief vortex and place 10 µl of the suspension into a 1.5 ml microfuge tube. Add 3 µl of p19 protein to the 10 µl beads suspension and incubate it on a bench top shaker, such as Orbits compact microtube shaker or an equivalent device for 10 –20 minutes at room temperature.

6. Binding of the Hybrid miRNA: RNA probe to the p19 beads: Prepare RNA Binding Buffer by adding the following into a 1.5 ml microfuge tube: 6 µl RNase free H_2O, 1 µl of 10X p19 Binding Buffer, 2 µl of 10X BSA (dilute from the 100X stock) and 1 µl of RNase inhibitor. After a brief mix, add the 10 µl RNA Binding Buffer to the RNA hybridization reaction to bring the final volume to 20 µl. Remove the supernatant from p19 chitin beads (from step 5) with a Magnetic Separation Rack. Add the entire 20 µl hybridization mix to resuspend the beads pellet. Incubate the RNA binding reaction by shaking for 1.5 hours at room temperature.

7. Removal of Unbound RNA: Excess probe and unbound RNA are removed by washing the p19 beads with BSA-wash buffer. BSA-wash buffer is prepared from the 25X wash buffer and 100X BSA stocks by dilution with sterile water to a final concentration of 1X wash buffer and 1X BSA. Heat up the 1X BSA-wash buffer to 37°C. Remove supernatant from RNA-p19 beads binding reaction using the Magnetic Separation Rack. Wash the beads pellet in 500 µl of 1X BSA-wash buffer and shake on a bench top shaker for 5 minutes at room temperature. Repeat the wash five times. To minimize any loss of the beads during the wash steps, allow the beads to settle to the bottom of the tube before using the magnetic rack. Beads are drawn to the side of the tube using the magnetic rack and the supernatant is carefully removed with a micropipetor.

8. Elution: After the last wash, remove as much of the supernatant as possible without touching the beads pellet. Add 40 µl of pre-warmed 1X p19 elution buffer to the beads pellet and incubate for 10 minutes at room temperature with shaking followed by another 10 minute incubation at 37°C. Carefully remove the supernatant containing miRNA:RNA probe hybrid from the microfuge tube using the magnetic rack. 5 to 10 µl of eluate is usually sufficient for analysis on a liquid scintillation counter or PAGE using a 20 % TBE gel.

Usage Notes:

Extensive washing is critical to reduce the background (non-specific binding of ssRNA probe to the chitin magnetic beads). We recommend the use of prewarmed (37°C) wash buffer and shaking of the beads during the incubation. Additional washing may be needed to reduce background.

The small amount of p19 beads used for miRNA detection require careful pipetting. Care in the removal of the supernatent is necessary to avoid loss of the beads.

References:

1. Silhavy, D. et al. (2002) *EMBO J.*, 21, 3070–3080.
2. Vargason, J.M. et al. (2003) *Cell*, 115, 799–811
3. Jin, J. et al. (2010) *Bio Techniques,* 48, XVII–XVIII.

Companion Products:

6-Tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

p19 siRNA Binding Protein
#M0310S 1,000 units
#M0310L 5,000 units

siRNA Marker
#N2101S 100 gel lanes

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