

p19 siRNA Binding Protein



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M0310S 003120514051

M0310S

RR 25°

1,000 units 10,000 U/ml Lot: 0031205

RECOMBINANT Store at -20°C Exp: 5/14

Description: The p19 siRNA Binding Protein (19 kDa) from the Carnation Italian Ringspot Virus (CIRV) plant binds siRNAs with nanomolar affinity(1). The dimeric protein binds 21 base siRNAs with a 2 base 3' extension and a 5' phosphate. The protein binds RNA in a size dependent and sequence independent manner. If the siRNAs are 4 bases longer, the affinity for the protein is reduced about 100-fold (2). When p19 siRNA Binding Protein is expressed in plants it suppresses RNA interference (3).

Source: p19 siRNA Binding Protein is cloned and expressed in *E. coli* as a fusion protein with an amino terminal MBP (maltose binding protein) and a carboxy terminal CBD (chitin binding domain).

0.00 0.05 0.1 0.2 0.5 1.0 1.5 2.0 3.0 M (µg)

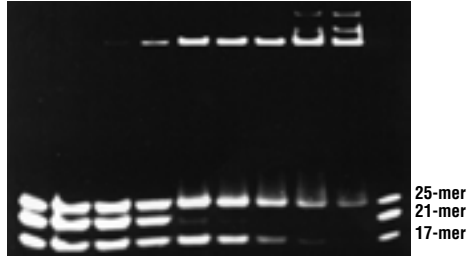


Figure 1: Size specific binding of siRNA by p19 siRNA Binding Protein. Three phosphorylated dsRNAs of 17,21 and 25 bases (30 ng each band) were mixed with increasing amount of p19 siRNA Binding Protein (0–3 µg) in a 20 µl reaction, and incubated at room temperature for 1 hour. The reaction was analyzed on a 20% polyacrylamide gel stained with ethidium bromide. Marker M is the siRNA Marker (NEB #N2101).

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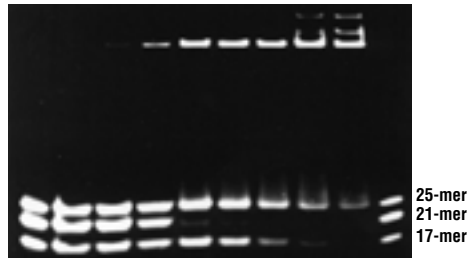


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Applications:

- High affinity binding of siRNAs
- Affinity purification of siRNA with chitin magnetic beads

Supplied in: 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5 mM DTT, 1 mM EDTA and 50% glycerol.

Reagents Supplied with Protein:

10X p19 siRNA Binding Buffer

1X p19 siRNA Binding Buffer:

20 mM Tris-HCl
100 mM NaCl
1 mM EDTA
1 mM TCEP
0.02% Tween-20
pH 7.0 @ 25°C

Unit Definition: One unit is defined as the amount of protein that binds to 10 ng of siRNA at 25°C in 1 hour.

Unit Assay Conditions: 1 unit of p19 siRNA Binding Protein was incubated with 20 ng of phosphorylated siRNA in 1X p19 siRNA Binding Buffer in a volume of 20 µl at 25°C for 1 hour.

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Quality Assurance: p19 siRNA Binding Protein contains no detectable DNases, RNases and phosphatases. The purified protein contains no detectable DNA or RNA as determined by ethidium bromide staining of an agarose gel.

Quality Control Assays

Exonuclease Activity: Incubation of 100 units of p19 siRNA Binding Protein for 4 hours at 37°C in 50 µl p19 siRNA Binding Buffer with a mixture of single and double-stranded [³H] *E. coli* DNA (200,000 cpm/µg) released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of 100 units of p19 siRNA Binding Protein for 4 hours at 37°C in 50 µl p19 siRNA Binding Buffer with 1 µg φX174 RF I DNA gave < 5% conversion to RF II.

Nuclease Activity: Incubation of 100 units of p19 siRNA Binding Protein for 16 hours at 37°C in 50 µl of p19 siRNA Binding Buffer with 1 µg λ DNA yielded a clear and sharp band on an agarose gel.

Protocol for isolation of siRNA from p19 siRNA Binding Protein using chitin magnetic beads:

The following protocol is recommended for the isolation of siRNA using 50 units of p19 siRNA

CERTIFICATE OF ANALYSIS

(See other side)

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Binding Protein. The composition of solutions that are not provided with p19 siRNA Binding Protein are provided. A magnetic rack (NEB #S1506, #S1509) is needed to separate the beads from solution.

1. Pretreatment of chitin magnetic beads with BSA (reduces non-specific binding):
 - a. Transfer a 200 µl suspension of chitin magnetic beads (NEB #E8036) into a sterile microfuge tube.
 - b. Pull the beads to the side of the tube using a magnetic rack and remove the supernatant.
 - c. Add 200 µl bead pretreatment buffer to the pellet (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM TCEP, 1 mg/ml BSA, pH 7.0 at 25°C).
 - d. Vortex and remove supernatant
 - e. Add 200 µl of fresh bead pretreatment buffer and rotate the tube at 4°C overnight.
- Note: The pretreated beads can be stored at 4°C for at least four months.
2. Binding of siRNA to p19 siRNA Binding Protein:
 - a. Add 50 units of p19 siRNA Binding Protein to RNA extract containing siRNAs or double stranded miRNAs in 1X p19 siRNA Binding Buffer.
 - b. Incubate for 1 hour at room temperature with shaking to form siRNA/p19 complex.

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3. Binding of siRNA/p19 complex to chitin magnetic beads:
 - a. Aliquot 20 µl of the pretreated beads suspension into a sterile microfuge tube.
 - b. Pull the beads to the side of the tube with a magnetic rack and remove the supernatant.
 - c. Add the siRNA/p19 complex to the pellet and mix by shaking or laying the tube on a magnetic stir plate for one hour at room temperature.
4. Remove unbound RNA:
 - a. Carefully remove supernatant containing unbound RNA.
5. Wash step:
 - a. Wash the beads with 500 µl of p19 siRNA Washing Buffer (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM TCEP, pH 7.0 at 25°C).
 - b. Vortex after wash.
 - c. Repeat three times. After third wash, remove as much buffer as possible without disturbing pellet.
6. Elution of siRNA:
 - a. Add 30–40 µl p19 siRNA Elution Buffer to bead pellet (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM TCEP, 0.5% SDS, pH 7.0 at 25°C).

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 - b. Vortex after wash.
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6. Elution of siRNA:
 - a. Add 30–40 µl p19 siRNA Elution Buffer to bead pellet (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM TCEP, 0.5% SDS, pH 7.0 at 25°C).

- b. Incubate at 37°C for 10 minutes.
- c. Mix on a stir plate for 10 minutes at room temperature.

Note: Elution step can be repeated and eluants can be combined if necessary.

Usage Notes:

p19 siRNA Binding Protein can selectively bind siRNAs in the presence of a 2,000 fold excess of other RNAs.

p19 siRNA Binding Protein does not bind ssRNA. Double-stranded 21 bp RNA with a 2-base overhang and a 5' phosphate binds the most efficiently. Double-stranded miRNAs with mismatched base pairs will bind with lower affinity.

Due to the difference in molecular weight between p19 siRNA Binding Protein and siRNA, a 10 to 20-fold excess of p19 siRNA Binding Protein is needed.

TCEP (Tris 2-carboxyethyl Phosphine) can be replaced with DTT (dithiothreitol) in the required solutions. However, if DTT is used, it needs to be added separately into the reaction.

References:

1. Silhavy, D. et al. (2002) *EMBO J.* 21, 3070–3080.
2. Vargason, J.M., et al. (2003) *Cell*, 115, 799–811.

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3. Qiu, W. et al. (2002) *Mol. Plant Microbe Interact.* 15, 269–280.

Companion Products:

Anti-MBP Monoclonal Antibody #E8032S	0.05 ml
#E8032L	0.25 ml

Anti-CBD Monoclonal Antibody #E8034S	0.05 ml
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Chitin Beads #S6651S	20 ml
#S6651L	100 ml

Chitin Magnetic Beads #E8036S	20 ml
#E8036L	100 ml

6-Tube Magnetic Separation Rack #S1506S	6 tubes
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12-Tube Magnetic Separation Rack #S1509S	12 tubes
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Notice to Buyer/User: The buyer/user has a non-exclusive license to use the vector for **Research Purposes Only**. Commercial use of this vector requires a license from New England Biolabs, Inc.

U.S. Patent No. 5,643,758
Appln. No. WO2007/120809

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