

## RNase Protection Assay

Marilyn J. Roossinck, Ph.D.

Synthesize an  $\alpha$   $^{32}\text{P}$ -labeled in vitro transcript complimentary to the target RNA, and an unlabeled transcript of the same sense as your target RNA (see In Vitro Transcription protocol # 28)

### Annealing

1 ml Buffer: 853  $\mu\text{l}$  deionized Formamide  
85  $\mu\text{l}$  5 M NaCl  
2  $\mu\text{l}$  0.5 M EDTA  
64  $\mu\text{l}$  0.5 M PIPES:KOH, pH 6.5

Combine 1  $\mu\text{l}$  probe (10,000 cpm)  
1  $\mu\text{l}$  viral RNA (~200 ng) (or, 2  $\mu\text{g}$  total RNA from infected plant)  
30  $\mu\text{l}$  Annealing buffer  
Incubate at 85°C for 2 min. then at 50-55°C for 2 to 3 hours

### RNase Digestion

Chill on ice: 920  $\mu\text{l}$  water  
60  $\mu\text{l}$  5 M NaCl  
10  $\mu\text{l}$  1 M Tris pH 7.5  
10  $\mu\text{l}$  0.5 M EDTA  
2  $\mu\text{l}$  RNase A (Sigma, 10 mg/ml)  
2  $\mu\text{l}$  RNase T1 (Calbiochem 2.5 Units/ $\mu\text{l}$ )  
Quick cool RNA samples on ice. Add 300  $\mu\text{l}$  digestion mix to each sample.  
Incubate at 15°C (also works at 30°C) for 1 hour. Add 20  $\mu\text{l}$  10% SDS, &  
10  $\mu\text{l}$  Pronase E (10 mg/ml) and incubate at 37°C for 15 minutes.  
Add 5-10  $\mu\text{g}$  carrier tRNA, extract with phenol:chloroform, and add 40  $\mu\text{l}$   
3 M NaOAc and 1 ml of ethanol to aqueous phase. Incubate at -20°C.

### Gel Electrophoresis

Analyze protected fragments by denaturing gel electrophoresis, either agarose (for larger fragments) or Urea/acrylamide.

Notes: Always use an unlabeled (+) transcript control to distinguish mismatches from A-U rich breathing.

See: Owen & Palukaitis, Virology 166:495, & Roossinck and Palukaitis, Mol. Plant Mic. Interact 3:188.