

S1 PROTECTIONS

Materials:

1. 10 X Hybridization Buffer
10 X = 4M NaCl
0.4M Pipes 6.8
0.02M EDTA
2. S1 Digestion Buffer
66 mM NaOAc
0.3 M NaCl
4mM ZnSO₄
3. tRNA 10 mg/ml
4. Formamide

Procedure:

1. Ethanol precipitate RNA sample, end labeled template, and carrier tRNA. Total amount of RNA should be 100 µg.
2. Resuspend pellet with 10 µl H₂O. Vortex.
3. Add 80 µl formamide, vortex.
4. Add 10 g 10X hybridization buffer. Vortex. Finger Spin.
5. Incubate at 68°C for 15 minutes. (65 to 75°C temp blot).
6. Transfer to 53°C and incubate for >4 hours. Upon transfer, tubes should not cool below 53°C. (Bring temp. block to water bath overnight.)
7. To each tube:
Remove from 53°C;
Add 300µl ice cold S1 buffer;
Quickly finger mix;
Place tube in ice;
Next tube, etc.
8. Add 0.5-1 µl S1 nuclease to each tube. If you have many samples you can add the S1 nuclease to the S1 buffer and add both of these in step 7.
9. Incubate 37°C, 60 minutes.
10. To stop digestion phenol extract.
11. Add 1ml ethanol (only) to precipitate. Dry pellets well (spin vac if necessary).
12. Resuspend pellets in 10 µl H₂O.
13. Remove an aliquot and add formamide dye. Because you are not loading your entire sample, you have the option to go back and re-digest your samples if you find that digestion was incomplete.
14. Incubate dye containing samples at 95°C for 5-10 minutes. Quick chill on ice.
15. Load on urea-polyacrylamide gel. Run 25 mA.
16. Fix gel with 7% acetic acid, 15 minutes.
17. Soak in H₂O or 1X TBE, 15-20 minutes.

18. Dry and expose.

Comments:

1. Originally, the amount of S1 nuclease required was titrated to digest 100 μg of total RNA. Conveniently, tRNA can be added at Step #1 to achieve this. However, if using large amounts of tRNA bothers you, there are two options:
 - a. In Step #1, leave out the carrier tRNA. Then, before phenol extracting in Step #10, add 20 μg tRNA, and
 - or--
b. As little as 20 μg carrier can be used in Step #1.
2. You must be absolutely sure that you are in template excess. There is an easy way, and a hard way, to confirm this.

Hard way: Do a series of protections with a constant amount of template and increasing amounts of test RNA. Over a range from 1 μg to 100 μg , your signal should increase proportionally.

Easy way: Load a 1:100 dilution of undigested template on gel along with your samples and compare the intensity of this band to those of your protected samples.
3. Sure fire way to make template: Short version. (See end labeling section for specific details.)

Digest 50-75 μg plasmid DNA. Phosphatase, kinase, etc. Resuspend final fragment in 100-200 μl TE. 1 μl is sufficient per S1. (200-400 cps per μl of template is ideal.)
4. For endogenous protections, use 5-10 μg of cytoplasmic RNA.
For protections of transfected genes, use 10-100 μg of RNA.