GAG assay

Safety precautions:

1. Do not inhale cetyl pyridinium chloride powder, it's irritating.
2. PPE: Wear protective clothing, including latex gloves and a lab coat. Saf-O will stain everything bright pink.

Materials:

Pipetmen
Pipet tips
Pipet Aid
Pipettes

Dot Blot apparatus

0.45 µm Nitrocellulose membrane (Bio-Rad 162-0115)
0.45 µm filters
1.5 ml tubes
Vortex
Water bath @ 37°C
Hole punch
Forceps- flat edge
Distilled water
Distilled water in squirt bottle
95% ethanol
Bucket
Clear tape
Microfuge tube racks (red only-for water bath)
96-well tissue culture plates (Falcon 353072)
Safranin O powder (Sigma S 8884)
10% Cetyl Pyridinium Chloride (CPC) (Acros Organic 226995000)
Chondroitin Sulfate (Associates of Cape Cod, Inc. 400675)
1M Sodium Acetate
Calf Thymus standard from previous DNA assay
Plate reader

Solutions

Safranin 0 reagent (0.02% safranin 0 in 50 mM sodium acetate, pH 4.8) Store at room temperature. Solution is stable for several months.
For 100 ml:
5 ml of 1 M sodium acetate (or 0.68 g of sodium acetate•3 H_2O)
About 85 ml of diH_2O
Adjust pH to 4.8 with acetic acid
Add 20 mg of Safranin 0 and dissolve
Bring volume to 100 ml with diH_2O
Millipore filter through 0.45 µm filter
10% CPC in H_2O Store at room temperature. Solution is stable for several months.
For 100 ml:
10 g of CPC
About 85 ml of diH_2O, warm to dissolve

Bring volume to 100 ml with diH_2O
Millipore filter through 0.45 µm filter.

Vacuum trap

Set up a 1000 ml Erlenmeyer flask with a 2-hole rubber stopper. Stick a length of plastic pipet through each hole; using Tygon tubing connect one to the dot blot apparatus and the other to a vacuum source. House vacuum should suffice.

Procedure:

1. Cut a piece of 0.45 µm nitrocellulose large enough to cover the necessary number of wells. Note: nitrocellulose is white. If the membrane you just cut is blue, you're using the backing paper. Prepare to have a really bad time with the assay. Laugh if you like, but I've seen it done. Several times. By the same person.

a. Moisten nitrocellulose in diH_2O

2. Assemble dot-blot apparatus
3. Add 250 µL of Safranin 0 reagent to wells4
4. Add blanks:
   - 25 µl for water blanks
   - 25 µl for papain blanks
   - Standards (25 µl, see below)
   - Samples (25 µl) to Safranin 0 reagent in wells (The reagent flows through the nitrocellulose, so only fill 5 wells at a time to avoid having too much reagent flow out of the wells by the time the sample is added.)
5. Let stand for about 1 min to allow precipitation
6. Cover unused wells
7. Tum on vacuum to collect precipitates
8. Rinse wells 2-3 times by filling with H_2O and sucking through
9. Remove nitrocellulose from dot-blot apparatus and air-dry on paper towel
10. Punch out dots from nitrocellulose with a hole-punch
11. Transfer the dots to 1.5-ml microfuge tubes
12. Add 1 ml of 10% CPC
    a. Incubate at 37°C for 20 min; vortex after 10 min
    b. Read absorbance at 536 nm

Standards 25 µl/well

CS-C	H_2O	0.2 mg/ml	1 mg/ml
1 µg	60 µl	15 µl	-
2 µg	45 µl	30 µl	-
3 µg	30 µl	45 µl	-
4 µg	15 µl	60 µl	-
5 µg	60 µl	-	15 µl
6 µg	57 µl	-	18 µl