

IMMUNO-DOT BLOT ASSAY

(Wright & Morton, 1989)

Introduction

This assay may be used as a positive or negative test for presence of glomalin but not for determining concentration. It also gives a long-term result, since the color on the nitrocellulose membrane does not fade (Fig. 1).

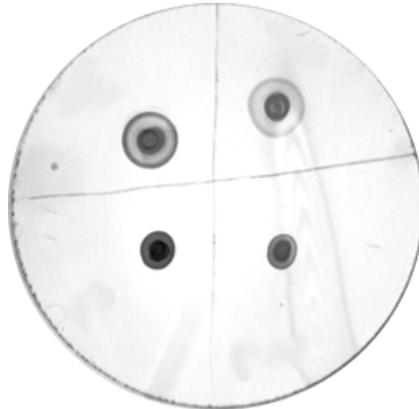


Figure 1. Assay conducted in June, 1998 on glomalin samples from a pot culture experiment.

Materials

0.2 um nitrocellulose membrane
Petri dish or dish for incubating the membrane
2% non-fat milk (2 g powdered milk/100 ml PBS)
PBS (phosphate buffered saline), pH 7.4
PBST (PBS with Tween 20), pH 7.4
1% BSA* (1 g BSA/100 ml PBS)
TBS (Tris buffered saline) (20 mM tris and 250 mM NaCl)
MAb32B11 antibody (diluted with PBS, typically 1 ml in 5 ml PBS)**
Biotinylated anti-mouse IgM (4.8 ul/6 ml 1% BSA)**
ExtrAvidin peroxidase (3.0 ul/ 6 ml 1% BSA)**
4-chlor-1-naphthol
Methanol
30% hydrogen peroxide
Dissecting needle or needle
Shaker or tilt table

*Make about 500-1000 ml of stock and dispense in 6 ml aliquots that will be frozen until needed.

**These are recommended concentrations. Chemicals obtained from different companies or with different lot numbers may need to be optimized for your conditions. Optimize for ELISA (see ELISA methodology) and use the same concentrations in this assay.

Methods

- 1) Divide membrane into small squares by etching lines into the membrane with a needle.
- 2) Place 1 ul of sample in a square and label by etching with a needle. (Typically the sample is undiluted, but if it is a concentrated sample, you may need to dilute it with PBS.)
- 3) Block membrane with 2% non-fat milk by shaking for 15 min.
- 4) Remove membrane, place on paper towel and dump out solution.
- 5) Add diluted MAb32B11 and incubate on shaker for 1 hr.
- 6) Remove antibody as described in step 4.
- 7) Add PBST, incubate on shaker for 5 min, and discard PBST as described in step 4. Repeat PBST incubation twice.
- 8) Incubate diluted biotinylated anti-mouse IgM with the membrane for 1 hr on shaker.
- 9) Remove IgM (see step 4), add PBST, incubate on shaker for 5 min, and discard PBST. Repeat PBST incubation twice.
- 10) Add diluted ExtrAvidin peroxidase and incubated for 1 hr on shaker.
- 11) Remove peroxidase, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice and once with TBS.
- 12) Develop with color developer (see below) until color is seen. Remove and dry. Store dry at room temp.

Color developer

- A. Make just prior to using.
- B. Mix 0.015g 4-chlor-1-naphthol in 5ml ice cold methanol.
- C. Immediately before use, add 25 ml TBS plus 15 ul of 30% hydrogen peroxide.