SOP: SP056

Con-A Blotting

Materials and Reagents:

1. Blotting Buffer (SOP R010)
2. 10X Blocking Buffer (10% BSA in TBST)
3. TBST (1.21 g Tris, 8.77 g NaCl, 0.5ml 100% Tween 80, q.s. to 1 L with MilliQ H_2O, pH 7.4).
4. TBS (1.21 g Tris, 8.77 g NaCl, q.s. to 1L with Milli-Q H_2O, pH 7.4)
5. HRP conjugated Concanavalin A
6. 4-Chloro-1-naphthol tablets
7. Methanol
8. Appropriate Glycosylated Substrate
1. Milli-Q H_2O
9. Chromatography paper 10 cm x 100 m (Whatman # 3030 672) also called "filter paper"
10. 0.2 µm Nitrocellulose Membrane (Biorad # 9004-70-0)
11. Blotting Cassette, complete with foam pads
12. Transfer tank
13. Transfer tank cover
14. Blotting cassette
15. Shaker Table
16. Power Supply with adapter
17. Completed SDS-PAGE gel (SOP SP007)

Protocol:

1._____ Run an SDS-PAGE gel according to SOP:SP007 with desired proteins or fractions.

2._____ While gel is running, prepare a transfer tank as described in SOP:SP011 for a Western Blot.

3._____ Once the gel is done running, place in transfer cassette on top of the nitrocellulose membrane, close the cassette, place in transfer tank, properly attach power source, and set voltage to 50V for 1 hour or 5-10V for 15 hours.

4._____ Once transfer is complete, turn off power supply and remove cassette and open.

5._____ Prepare 1X blocking buffer by making a 1:10 dilution of 10% BSA in TBS in a small blotting container.

6._____ Transfer the membrane to the small blotting chamber and discard of gel and filter paper.

7._____ Place blotting chamber on shaker table and let incubate for at least 1 hour.

8._____ While membrane is incubating, prepare the HRP labeled Con-A by diluting 50 µl of pre-alliquotted HRP-Con-A (stored in the general use -20°C) in 10 ml TBS.

9._____ Pour off blocking buffer and rinse the membrane three times briefly with TBST, then for 5 minutes on the shaker table.

10._____ After rinsing, apply the HRP-Con-A and let incubate overnight at 4 degrees.

11._____ At the end of the incubation, prepare the developer of 4-Chloro-1-naphthol, by dissolving one tablet in 10 ml methanol.

12._____ Add 2 ml of the methanol/4-Chloro-1-naphthol solution to 8ml TBS (Note 1). Directly before use, add 5 µl of hydrogen peroxide.

13._____ Pour of the HRP-Con-A and rinse membrane three times briefly with TBS, then for 5 minutes on the shaker table.

14._____ After rinsing, apply the developer solution and place on shaker table watching for blot to develop.

15.____ Once your band of interest appears, pour off the developing solution and rinse with Milli-Q H_20 several times to stop the reaction.

16._____ Place the blot on a paper towel and fold the towel to cover the blot.

17._____ Once dry, the blot can be placed in a notebook, or scanned.

Notes

1. Avoid using TBST as it causes the 4-Chloro-1-naphthol to precipitate out.