PROTOCOL FOR WESTERN BLOTTING

Quan Kang 7/10/03; Updated by Ying Peng, 5/17/04

1. Run the samples on SDS-polyacrylamide gels (**Note**: If you want your gel looks pretty, **load the same amount of Laemmli sample buffer in the empty lanes**) set at 180V/30mA. If two gels are running at the same time, use 180V/60mA.
2. Cut and mark the Immobilon-P membrane (Millipore). Soak it in methanol for about one minute and then wash it with distilled water (do not let water drop directly on the membrane).
3. Equilibrate the membrane and blot papers (two for each membrane) in transfer buffer (store at room temperature).
4. Assemble transfer sandwich: transfer plate, membrane, SDS-PAGE gel, and lid. Transfer at 20V/250mA for 45 minutes at room temperature.
5. Block the membrane (rocking) with SuperBlock DryBlend Blocking Buffer in TBS (Pierce) for one hour at room temperature.
6. Probe the membrane with the first/primary antibody diluted with SuperBlock DryBlend Blocking Buffer (1:1000-1:2000) and rock for one hour at room temperature.
7. Wash the membrane with TBST 5 minutes X 3 (rocking).
8. Dilute the secondary antibody conjugated with horseradish peroxidase (Pierce) with TBST (1:5,000- 1:10,000) and incubate the membrane for 30 minutes.
9. Wash the membrane with TBST 5 minutes X 3.
10. Mix the reagents of SuperSignal West Pico or West Femto chemiluminescent substrate kit (1:1) - totally 3-4 ml is enough (cover with foil).
11. Soak the membrane in the detection solution for 30-60 seconds.
12. Take pictures with the membrane facing down in the Image Station (**Note**: you may try a 20 sec. quick exposure first, and adjust exposure time accordingly).

TBST (Tris-Buffered Saline-Tween-20):

10mM Tris-HCl (pH7.5)
150mM NaCl
0.05% Tween-20 (added after all other reagents and water are mixed)

Transfer Buffer:

800ml Methanol
12.12g Tris
57.63g Glycine
Total: 4L