Mouse Sera Analysis Protocol (revised 5/13/05):

1. Run 10 ug of human 3'HIP1 protein, gel 10 % : after run, cut the gel
2. Transfer 90 min (membrane 10x7.5 cm) : after transfer, write the gel limit on membrane
3. Blocking O/N in TBS-T, 5 % milk, 5 % normal goat serum
4. Prepare samples : 65 uL TBS-T/milk + 5 uL sample serum (in duplicate – 130ul TBST milk + 10 ul sample serum)
   Controls: 1B11 (2mg/mL) dilutions in milk (1:10,000, 1:20,000, 1:40,000, 1:80,000)
5. Clean the miniblotter plates (aspirate dust or wash off).
6. Position the membrane against the channels – line up ladder with channels
7. Cover the membrane with a single layer of plastic wrap, and then with foam cushions.
8. Assemble the miniblotter plates, very tight.
9. Aspirate excess liquid.
10. Load the channels with 60 uL TBS-T/milk to avoid the drying of the membrane.
11. Aspirate liquid from one channel and load 60 uL of sample, avoiding air bubbles.
12. Incubate 2h at RT on a rocking platform, with slow rocking speed.
13. Remove all samples by aspirating.
14. Wash the membrane with 300 ml TBS-T using the manifold.
15. Cut the membrane (in order to use a small tray)
16. Wash the membrane quickly 3x with ddH2O and 1h in TBS-T (3-6 changes)
17. Add 1/10,000 secondary antibody (Goat anti mouse) in TBST/milk for 1 hour at RT.
18. Wash the membrane quickly 3x ddH2O and 1 h in TBST (3-6 changes).
19. Develop with supersignal Pierce ECL (1.5 min. incubation) – 10 sec-3 min exposure.
20. Wash gently and dry the miniblotter and the manifold.