Affinity Absorption of Hamster Anti-Tg Ascites

1. Dissolve affinity purified SV40 Tg (~200ng) in 5ml Western blot transfer buffer, vacuum spot onto 1.5x3cm nitrocellulose filter.

2. Block with 3%BSA in 10mM TrisCl pH8.0, 150mMNaCl, 0.2% Tween-20(TBST), 30min RT. Incubate with 20ml of 1/20 dilution of hamster ascites in TBST, RT 1hr with agitation.

3. Wash 4x5min with TBST with agitation. Elute antibody with 4-5ml 10mM glycine-HCl pH2.7 in 30 seconds. Immediately neutralize eluate by adding 1/10 volume of 1.5M TrisHCl pH8.8. Put filter back to TBST, wash 2x5min, reblock 20min with 3%BSA, reabsorb diluted ascites for 1hr, repeat elution.

4. Both filter and diluted ascites can be repeatedly used for 3-4 times. Wash filter 2x5min with TBST, air dry O/N, store at 4°c, can be reused with reduced efficiency.

5. Pool eluate and dialyse in PBS O/N at 4°c. Concentrate to about 0.6ml with Centricon-10. Add 1/10 volume normal goat serum, store frozen at -20°c in small aliquots.

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