

Ren Lab Peptide Dot Blot Antibody Specificity Screening

Dot Blot Protocol Revised 080609

1. Print 1 μ l peptide onto nitrocellulose membrane with use of Biomek robot, in 96 well format. (For a total concentration of 0.02 μ g and/or 0.2 μ g peptide per dot – two amounts per peptide.)

2. Allow peptides to air dry on membrane.
3. Block membrane for 1hr with 5% nonfat milk in TBS-T.
4. Wash once with TBS-T. (Can be frozen for later use.)
5. Incubate with primary antibody in 5% nonfat milk in TBS-T overnight at 4°C.
6. Wash three times with TBS-T, five minutes each.
7. Incubate with secondary antibody in 5% nonfat milk in TBS-T, for 1 hour at room temperature.
8. Wash three times with TBS-T, five minutes each.
9. Develop with ECL.