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NBP2-11055PEP Protocol

Antibody Competition Protocol for DPYSL4 Protein (NBP2-11055PEP)

In this assay the antigen binding sites (Fab) of a particular antibody is allowed to bind to homologous antigenic peptide in several hundred molar excess ratio of immunoglobulin to peptide. Since the antigenic peptide has higher affinity for Fab site than the full length native proteins, the antigen binding site is blocked thus antibodies are unable to bind to the specific sites on either native, partial denatured or completely denature antigen on sections, in solution or on membrane blots.

Before starting, standardize the conditions for Western blot, immunoprecipitation or immunohistochemistry using the relevant antibody. Conditions include: volume of antibody used; dilution factor, final volume, incubation time, washing conditions etc.Once conditions have been standardized, repeat the same protocol in duplicate using blocked and unblocked antibody.

Begin the competition assay by preparing the blocked antibody-peptide solution:

1. Take the same volume of antibody as previously standardized. Let us assume 10 uL in 15 mL.
2. Add approximately 1:200 moles of excess peptide (peptides are generally 2200 dalton). This will be equivalent of approximately 60-70ul of peptide solution (original peptide concentration is 2.5mg/ml).
3. Mix 10 uL of antibody with 60-70 uL of peptide and make up the volume to 200 uL using DiluOBuffer.

Compare the above blocked antibody-peptide solution with control antibody:

1. Take same amount of antibody as used in the blocked antibody-peptide solution and add 60-70 ul of PBS and make up the volume to 200 ul using DiluOBuffer.
2. Once both blocked antibody-peptide solution and the control antibody solution have been made, incubate both solutions at 4 degrees C overnight on a rotating mixer. Centrifuge both tubes at 12,000-14000 xg for 1-2 minute at 4 degrees C. A small amount of precipitate may accumulate in the blocked antibody-peptide solution due to antibody-antigen complex formation. If this occurs, carefully remove the precipitate and use only the supernatant.
3. Dilute both solutions in 1X DiluOBuffer to the final dilution volume of 15 mL. The antibody-peptide solution will not give any labeling or will have significantly reduced labeling while the Control Antibody solution should provide standard results.