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NB600-444 Protocol

Serum protocol for Ethenocytidine Antibody (NB600-444)

Ethenocytidine Antibody (6F5): https://www.novusbio.com/products/ethenocytidine-antibody-6f5_nb600-444
Competitive ELISA

I Coating of Plates

DNA coating: DNA is dissolved in PBS at appropriate concentration. 0.1 ml is added/well and plates put in 37 degrees Celsius incubator to evaporate overnight. Alternatively, plates can be coated with a 2-fold higher concentration of DNA for 2 hrs at 37 degrees Celsius then used. Column 1 is not coated. These well will not be used for the assay (no blocking, no antibody and no secondary antibody) but will have substrate added for blanking the reader. Plates are stored in the refrigerator.
Protein coating: Proteins are dissolved in PBS at the appropriate concentration. 0.1 ml is added/well and plates put in 37 degrees Celsius incubator to evaporate overnight. Column 1 is again not coated. Plates are stored in the refrigerator.
An alternate protein coating condition is to dissolve the protein in 0.1 M sodium carbonate buffer pH 9.6. 0.1 ml is added/well and the plates are refrigerated for several hours or overnight. They cannot be used after 3 days.
1 M solution 1.59 g Na_2CO_3 + 2.93 g $NaHCO_3$/100ml

II Assay

1. Label assay sheet and determine which rows are to be used. Row 1 (A-H) is not used; it will be used to blank the spectrophotometer. Avoid using the outer rows if possible (i.e.12A-H, H 1-12 and A 1-12.

2. Wash plate with wash buffer containing PBS-Tween and NaN_3 3 x on each side (right side up and upside down). Shake out onto paper towel.

3. Add 0.2 ml/well of 1% FCS in wash buffer to block non specific binding. Solution of FCS should be made fresh.

4. Incubate 1 hr.

5. Preparation of inhibitor series (during incubation of plate with FCS). Calculate appropriate concentrations to give desired fmol/well=fmol/0.05 ml. Make serial dilutions by adding PBS or CT DNA to tubes followed by competitor.

6. Prepare antibody in 1% FCS washing buffer.

7. At end of incubation period, shake out solution from plate and tap onto paper towel to dry.

8. Add 0.05 ml of competitor to each well followed by 0.05 ml of diluted antibody. Be sure to run all controls including zero (no competitor), minus Ab (no antigen specific antibody but secondary antisera) and positive and negative controls.

9. Incubate for 90 min at 37 degrees Celsius.

10. Wash the plate with washing buffer 3 times on each side. Tap onto paper towels.

11. Secondary antisera - Use goat anti-mouse IgG-alkaline phosphatase for monoclonals and anti rabbit for polyclonals. Dilute as appropriate and add 0.1 ml/well.

12. Incubate for 90 min at 37 degrees Celsius.

13. Wash with wash buffer 3 x each side. Tap onto paper towel.

14. Wash plate 2 times with 0.01 M diethanolamine using the was bottle and covering the well completely each time. Tap onto paper towel. This step removes phosphate buffer which inhibits alkaline phosphatase activity.

15. Prepare the substrate - 2 tablets 95 mg/tablet) Sigma 104 in 10 ml 1 M diethanolamine, pH 8.6. Final concentration 1 mg/ml. Avoid physical contact of skin with the tablets since skin contains alkaline phosphatase. Add 0.1 ml/well

16. Incubate at 37 degrees Celsius and read absorbance at 405 nm. The absorbance of the 0 fmol standard should be between 0.5 and 1. Values above 2 are not usable since the reader may not be linear in this range.

Rinse water - One liter of H_2O + 2 ml 10% NaN_3

Wash buffer - One liter of 1 x PBS + 500 ul Tween 20 + 2 ml 10% NaN_3

Blocking buffer - Wash buffer + 1% FCS