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NBP1-97919 Protocol

Sandwich ELISA for ChABC Methods (NBP1-97919)

ChABC Antibody Pair: https://www.novusbio.com/products/chabc-antibody-pair_nbp1-97919
Capture Plate Preparation (solid-phase IgG)

1. Dilute the Capture Antibody, NBP1-96142, to 2 ug/ml in PBS. Add 100 ul/well to all wells of a 96-well plate. Cover plate with adhesive film. Let adsorb for 2 hr at room temp (or overnight at 4C).

2. Flick contents into a sink and flush the entire plate thoroughly with purified water, ensuring that all wells are filled. Flick contents and wash the wells this way in rapid succession three times. After the last wash, remove any remaining water by slapping the plate into clean paper towels and then wiping the outer surfaces.

3. Block the wells by adding 100 ul/well of Reagent Diluent (PBS containing 1% BSA) and incubate at room temp for 1 hr.

4. Aspirate Blocking Buffer and add 200 ul/well PBS + 0.1% Triton X100. Incubate for 10 min at room temperature. Aspirate immediately before proceeding to the next step.

Assay Procedure

1. Add 100 ul/well of test samples or ChABC standards diluted in Reagent Diluent. Cover with adhesive film and incubate for 2 hr at room temp with gentle mixing on a rocking platform.

2. Aspirate and wash 3 times with PBS + 0.1% Triton X100 (200 ul/well), aspirating between 5 min washes.

3. Diluted Detection Antibody, NBP1-96141B, to 1.0 ug/ml in diluted in Reagent Diluent. Add 100 ul/well of the working dilution to all wells. Cover with adhesive film and incubate for 1 hr at room temp with gentle mixing on a rocking platform.

4. Aspirate and wash as in Step 2.

5. Diluted Detection Reagent, Streptavidin/HRP, 1:4000 in Reagent Diluent. Add 100 ul/well of the working dilution to all wells. Cover and incubate for 30 min at room temp on a rocking platform. Avoid exposure to direct light.

6. Wash 5 times with PBS + 0.1% Triton X100 (200 ul/well) for 5 min each, followed by a final brief rinse in running purified H_2O (to remove Triton and any stray reagents). Flick the plate to remove water, slap several times into clean paper towels and wipe clean the underside of wells.

7. Add 150 ul/well of Colorimetric Substrate solution, OPDA (0.05%) in 0.1 M Phosphate/Citrate buffer pH 5.0 + H_2O_2 (0.02%). Incubate on a rocking platform shaker at room temp. (When developing, protect from the light by covering with a dark towel or foil.)

8. After 10 min, or when developed sufficiently, stop colorimetric reaction by adding 50 ul/well of 2M H_2SO_4. (Sufficient development is achieved when 5 ng/ml ChABC has a visible color (>0.1 OD)

9. Tap the plate for 10 sec to mix (or use mixing function in microplate reader). Read the product absorbance using a microplate spectrophotometer with a 492 nm filter. Take readings within 10 min of adding the H_2SO_4.

Stocks, Materials and Solutions

Capture Antibody: NBP1-96142, ChABC Antibody (6A12)

Detection Antibody: NBP1-96141B, ChABC Antibody (1E10) [Biotin conjugated]

ChABC Standard: Sigma #C3667. A useful standard curve range is 1-100 ng/ml. The lower detection limit is ~1 ng/ml. Starting with the Working solution at 100 ng/ml, make a standard curve with 8 concentration points (100-0.75 ng/ml) using 2-fold serial dilutions in PBS+1%BSA. Include 0 ng/ml ChABC control wells in PBS+1%BSA.

Note: Lyophilized Chondroitinase ABC (Sigma #C3667) is supplied at 5 Units/vial. Reconstituted with 200 ul sterile H2O (it contains no preservative or protein carrier.). Perform Bradford Protein assay using BSA as a standard to determine protein content independent of the protein content information provided on the product label. Aliquot and store at -80C (or -20C). From this frozen stock, prepare a Working dilution at 100 ng/ml in PBS+1% BSA. This diluted Working solution must be aliquoted and stored frozen.

Streptavidin/HRP: Sreptavidin conjugated to horseradish-peroxidase (DakoCytomation, #P0397). Dilute to working concentration of 1:4000 in PBS+1%BSA.

Wash Buffer: PBS + 0.1% Triton X100

Reagent Diluent: 1%BSA in PBS (Bovine serum albumin, Fraction V, Sigma A-9647). Filter through 0.45 um membrane.

Substrate Solution: OPDA (o-phenylenediamnine) (Sigma #P1526) at 0.05% in 0.1 M Phosphate/Citrate buffer pH 5.0 (+/- 0.5) + 0.02% H2O2.
(Bring stocks to room temp to solubilize before making the buffer.)
Buffer: 5 ml 0.2 M dibasic sodium phosphate + 5 ml 0.1 M citric acid + 10 ml dH2O, mix add 10 mg OPDA, mix until dissolved and then add 13 ul H2O2 (30%). Use immediately.

Stop Solution
2 M (10%) H2SO4