

Product Datasheet

ChABC Antibody Pair NBP1-97919

Unit Size: 2 Vials

Store at 4C. Do not freeze.

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NBP1-97919**ChABC Antibody Pair****Product Information**

Unit Size	2 Vials
Concentration	Concentration of individual antibodies may be found on the vial label. If unlisted please contact technical services.
Storage	Store at 4C. Do not freeze.
Buffer	NBP1-96141B: PBS with No Preservative NBP1-96142: Tris-glycine, 150mM NaCl pH 7.5 and 0.05% Sodium Azide

Product Description

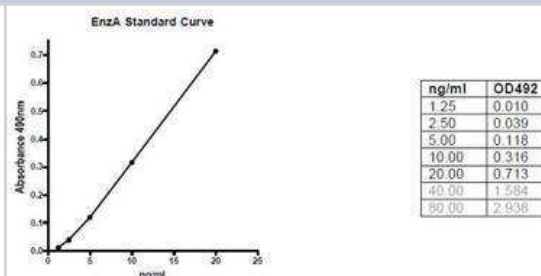
Species	Non-species specific
Kit Components	NBP1-96142: ChABC Antibody (6A12), NBP1-96141B: ChABC Antibody (1E10) [Biotin]

Product Application Details

Applications	ELISA, Sandwich ELISA
Recommended Dilutions	ELISA 1-2 ug/ml, Sandwich ELISA 1-2 ug/ml
Application Notes	ELISA data: Capture Antibody ChABC/6A12 [NBP1-96142] was bound to a 96-well ELISA plate and serial dilutions of ChABC were added in physiological buffer. Bound ChABC was labeled using Detection Antibody biotinylated ChABC/1E10 [NBP1-96141B]. Bound Detection Antibody was labeled with avidin-HRP and the assay developed with chromagen OPDA/ H2O2.

Images

ELISA: ChABC Antibody Pair [NBP1-97919] - Standard curve for serial dilutions of ChABC. See Notes for additional information.



Procedures

Sandwich ELISA for ChABC Methods (NBP1-97919)

ChABC Antibody Pair:

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 H₂O (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 + H₂O₂ (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 H₂SO₄. (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 H₂SO₄.

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: Streptavidin 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





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Products Related to NBP1-97919

NBP1-96141	ChABC Antibody (1E10)
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Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Antibody Pairs are guaranteed for 6 months from date of receipt.

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