Human NAP-2 (PPBP) ELISA Kit

Catalog Number EHPPBP (96 tests)

Rev. 6

Product description

The Human NAP-2 (PPBP) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human NAP-2 in serum, plasma, and cell culture media.

Contents and storage

Upon receipt, store at 2-8°C for 6 months or -20°C for 1 year.

Components	Cat. No. EHPPBP (96 tests)
Human NAP-2 Antibody Coated wells, 96-well plate	1 plate
Human NAP-2 Biotin Conjugate	2 vials
Human NAP-2 Standard, recombinant human NAP-2	2 vials
Wash Buffer Concentrate (20X)	25 mL
Assay Diluent A (contains 0.09% sodium azide)	30 mL
Assay Diluent B (5X)	15 mL
Streptavidin-HRP (200X)	0.2 mL
TMB Substrate	12 mL
Stop Solution	8 mL
Adhesive Plate Covers	2

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal axides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Materials required but not supplied

- Distilled or deionized water
- Microtiter plater reader with software capable of measuring at 450 nm
- Plate washer-automated or manual (manifold dispenser)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Procedural guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* at **thermofisher.com** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Wash Buffer

1. Allow Wash Buffer Concentrate (20X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 20 mL of the Wash Buffer Concentrate into 380 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within one month.

Prepare diluent

- Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

Prepare biotin conjugate

1. Briefly spin down the biotin conjugate before use.
2. Add 100 µL of 1X Assay Diluent B into the vial to prepare a biotin conjugate concentrate.
3. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days).
4. The biotin conjugate concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 2 of ELISA procedure.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

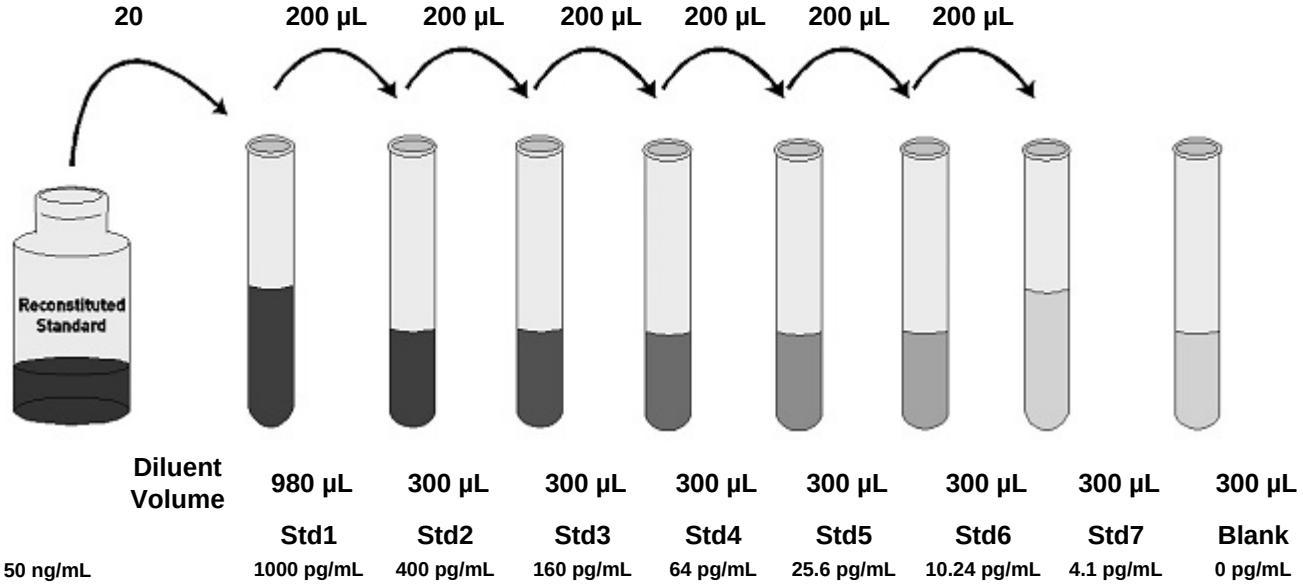
Pre-dilute samples

- Assay Diluent A should be used for dilution of serum and plasma samples. 1X Assay Diluent B should be used for dilution of cell culture supernatant samples.
- Dilute **serum and plasma** 1,000 - 20,000-fold.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly spin down a vial of lyophilized standard.
2. Add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into the lyophilized standard vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by gentle mixing. Add 20 µL NAP-2 standard from the vial of reconstituted standard, into a tube with 980.0 µL Assay Diluent (A or B) to prepare a 1,000 pg/mL stock standard solution. Pipette 300 µL Assay Diluent (A or B) into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent (A or B) serves as the zero standard (0 pg/mL).



Prepare 1X Streptavidin-HRP solution

Note: Prepapre the Streptavidin-HRP within 15 minutes of usage.

1. Briefly spin the Streptavidin-HRP and pipette up and down to mix gently before use, as precipitates may form during storage.
2. Dilute Streptavidin-HRP 200-fold with 1X Assay Diluent B.
3. Do not store diluted solution for future use.

Perform ELISA (Total assay time: 4 hours and 45 minutes)

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use.

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

1 Bind antigen

a. For the standard curve, add 100 µL of standards to the appropriate wells (see Dilute standards). For samples, add 100 µL of diluted samples (see Dilute samples) to the wells.

b. Cover wells and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

c. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

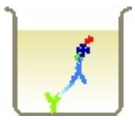
2 Add biotin conjugate

a. Add 100 µL of prepared biotin conjugate (see Prepare biotin conjugate) to each well.

b. Incubate for 1 hour at room temperature with gentle shaking.

c. Discard the solution. Repeat the wash as in step 3.

3 Add Streptavidin-HRP



a. Add 100 µL of prepared Streptavidin-HRP solution (see Prepare Streptavidin-HRP solution) to each well.

b. Incubate for 45 minutes at room temperature with gentle shaking.

c. Discard the solution. Repeat the wash as in step 3.

4 Add TMB substrate

a. Add 100 µL of TMB Substrate to each well. The substrate will begin to turn blue.

b. Incubate for 30 minutes at room temperature **in the dark** with gentle shaking.

5 Add stop solution

Add 50 µL of Stop Solution to each well. Tap the side of the plate gently to mix. The solution in the well changes from blue to yellow.

Read the plate and generate the standard curve

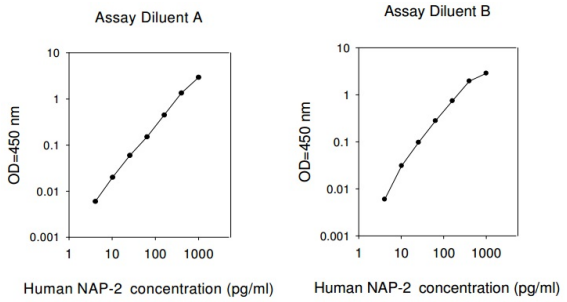
1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and control from the standard curve. Multiple value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than that of the higest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

These standard curves are for demonstration only. A standard curve must be run with each assay.



Intra-assay precision

To determine intra-assay precision, two standard curves and 3 samples for each standard curve are run. The standard curve concentration points as well as the samples are tested in duplicates on a single plate. Two different concentration values are obtained for each sample, using the two separate standard curves. The two concentration values for each sample is compared to each other using the CV% calculation.

Intra-Assay CV%: <10%

Inter-assay precision

To evaluate inter-assay precision, the second standard curve is tested on a separate plate along with the second set of samples.

Inter-Assay CV%: <12%

Recovery

Sample Type	Average % Recovery	Range (%)
Serum	93	82-102
Plasma	93	84-103
Cell Culture Media	95	84-103

Specificity

This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

Linearity of dilution

The serum, plasma, and cell culture media samples were spiked with recombinant human NAP-2, serially diluted in sample diluent and evaluated. Observed values were compared to expected values to calculate percent recovery and demonstrate the dilution linearity of the assay.

Sample Type	Average % Expected		Range (%)	
	1:2 Dilution	1:4 Dilution	1:2 Dilution	1:4 Dilution
Serum	93	95	83-102	84-104
Plasma	92	94	82-102	83-102
Cell Culture Media	95	102	83-103	85-104

Sensitivity

The minimum detecable dose of human NAP-2 is 8.5 pg/mL. This was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Limited product warranty

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Product label explanation of symbols and warnings