

Human NAP-2 ELISA Kit

User Manual

ELISA Kit Protocol

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I. INTRODUCTION

NAP-2 (neutrophil-activating protein-2) which is a protein with a length of 70 amino acids, belongs to the family of chemotactic cytokines. The expression of NAP-2 is induced in human monocytes by treatment with bacterial endotoxins. It stimulates the release of elastase from neutrophils and shows some of the activities of IL8. NAP-2 interacts with the NAP-1 receptor and binds to one type of receptors that also binds IL8 and related factors.

The Human NAP-2 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human NAP-2 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human NAP-2 coated on a 96-well plate. Standards and samples are pipetted into the wells and NAP-2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human NAP-2 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of NAP-2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. REAGENTS

1. NAP-2 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human NAP-2.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials of recombinant human NAP-2.
4. Assay Diluent A (Item D): 30 ml, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.

5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Detection Antibody NAP-2 (Item F): 2 vial of biotinylated anti-human NAP-2 (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 8 μ l of 10,000x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

III. STORAGE

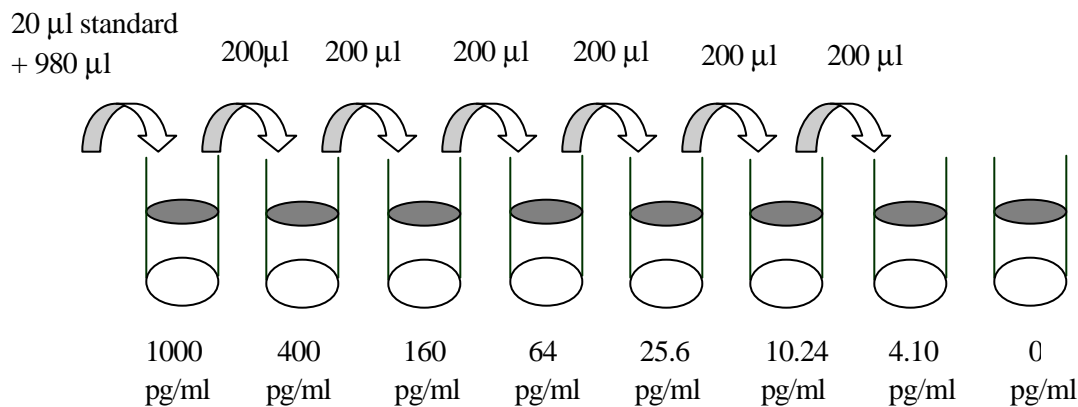
May be stored for up to 5 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells and reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge of zip-seal.

IV. ADDITIONAL MATERIALS REQUIRED

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- 2 Precision pipettes to deliver 2 μ l to 1 ml volumes.
- 3 Adjustable 1-25 ml pipettes for reagent preparation.
- 4 100 ml and 1 liter graduated cylinders.
- 5 Absorbent paper.
- 6 Distilled or deionized water.
- 7 Log-log graph paper or computer and software for ELISA data analysis.
- 8 Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Preparation of standard: **Briefly spin the vial of Item C.** Add 400 μl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 20 μl NAP-2 standard from the vial of Item C, into a tube with 980.0 μl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) to prepare a 1,000 pg/ml stock standard solution. Pipette 300 μl Assay Diluent A or 1x Assay Diluent 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 1x Assay Diluent B serves as the zero standard (0 pg/ml).



3. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants and urine.
4. Assay Diluent B should be diluted 5-fold with deionized or distilled water.

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 10,000-fold with 1x Assay Diluent B.

*For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 **ml** of HRP-Streptavidin concentrate into a tube with 198.0 **ml** 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 120 **ml** of prepared 100-fold diluted solution into a tube with 12 ml 1x Assay Diluent B to prepare a final 10,000 fold diluted HRP-Streptavidin solution.*

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ l of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C.
3. Discard the solution and wash 4 times with 1x Wash Solution (200 μ l each).

4. Add 100 μl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature.
5. Discard the solution and wash 4 times with 1x Wash Solution (200 μl each).
6. Add 100 μl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature.
7. Discard the solution and wash 5 times with 1x Wash Solution (200 μl each).
8. Add 100 μl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark.
9. Add 50 μl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.



2. Add 100 μl standard or sample to each well.
Incubate 2.5 hours at room temperature or over night at 4°C.



3. Add 100 μl prepared biotin antibody to each well.
Incubate 1 hour at room temperature.



4. Add 100 μl prepared Streptavidin solution.
Incubate 45 minutes at room temperature.



5. Add 100 μ l TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.



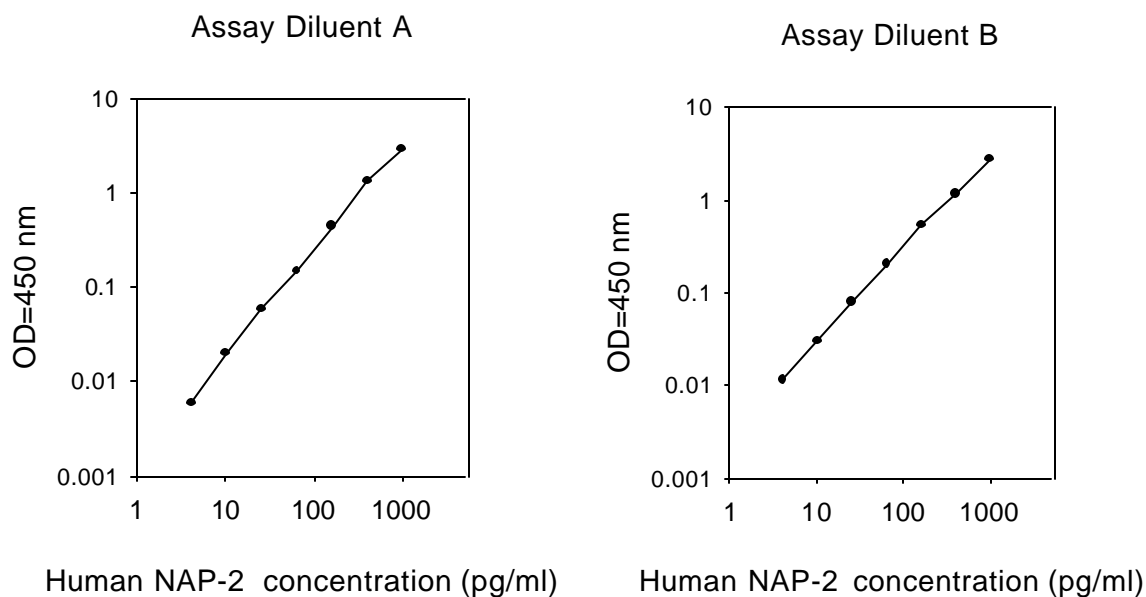
6. Add 50 μ l Stop Solution to each well.
Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

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



B. SENSITIVITY

The minimum detectable dose of NAP-2 is typically less than 8.5 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of human NAP-2 into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	92.76	82-102
Plasma	93.45	83-103
Cell culture media	95.28	84-103

D. LINEARITY

Sample Type	Serum	Plasma	Cell Culture Media
1:2 Average % of Expected Range (%)	93 83-102	92 82-102	95 83-103
1:4 Average % of Expected Range (%)	95 84-104	94 83-102	102 85-104
1:8 Average % of Expected Range (%)	96 85-104	94 83-108	97 84-103

E. REPRODUCIBILITY

Intra-Assay: CV<10%

Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (*e.g.*, human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 α , IL-1 β , 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, IL-309, IP-10, G-CSF, GM-CSF, IFN- γ , Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 α , MIP-1 β , MIP-1d, PARC, PDGF, RANTES, SCF, TARC, TGF- β , TIMP-1, TIMP-2, TNF- α , TNF- β , TPO, VEGF).

X. REFERENCES:

1. Leonard EJ et al, Chemotactic activity and receptor binding of neutrophil attractant/activation protein (NAP-1) and structurally related host defense cytokines: interaction of NAP-2 with the NAP-1 receptor. *Journal of Leukocyte Biology* 49: 258-65 (1991).
2. Moser B et al, Neutrophil-activating peptide 2 and gro/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. *Journal of Biological Chemistry* 266: 10666-71 (1991).
3. Walz A et al, Effects of neutrophil-activating peptide NAP-2, platelet basic protein, connective tissue-activating peptide III, and platelet factor 4 on human neutrophils. *Journal of Experimental Medicine* 170: 1745-50 (1989).

XI. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	<ol style="list-style-type: none"> 1. Inaccurate pipetting 2. Improper standard dilution 	<ol style="list-style-type: none"> 1. Check pipettes 2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	<ol style="list-style-type: none"> 1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution 	<ol style="list-style-type: none"> 1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	<ol style="list-style-type: none"> 1. Inaccurate pipetting 	<ol style="list-style-type: none"> 1. Check pipettes
4. High background	<ol style="list-style-type: none"> 1. Plate is insufficiently washed 2. Contaminated wash buffer 	<ol style="list-style-type: none"> 1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	<ol style="list-style-type: none"> 1. Improper storage of the ELISA kit 2. Stop solution 	<ol style="list-style-type: none"> 1. Store your standard at $<-20^{\circ}\text{C}$ after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

Notes:

