HUMAN CXCL7/NAP-2 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CXCL7/NAP-2 CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN CXCL7/NAP-2 ELISA KIT
Catalog No.	SK00434-01A
Lot No.	
Formulation	96 T
Standard range	31.25-8000 pg/mL
Sensitivity	15 pg/mL
Sample require	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, Plasma
Specificity	Human CXCL7 only
Intra-assay Precision	4-6%
Inter-assay Precision	4-8%
Storage	2-8°C for 1 month. See page 2-3 for more information

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human CXCL7 /NAP-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human CXCL7 in serum and plasma. It contains recombinant human CXCL7 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human CXCL7. Results obtained with naturally occurring CXCL7 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human CXCL7.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CXCL7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CXCL7 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for CXCL7 is added to the wells. Following a wash to remove any unbound antibody reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CXCL7 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CXCL7 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against CXCL7.	434-01A-01	1 plate
CXCL7 Standard – 8000 pg/vial of recombinant human CXCL7 in a buffered protein base with preservatives; lyophilized.	434-01A-02	1 vial
Detection Antibody – 105 µL/vial, 100-fold Concentrate of Biotinylated polyclonal antibody against CXCL7 with preservatives; lyophilized.	434-01A-03	1 vial
Positive Control – one vial of recombinant human CXCL7, lyophilized	434-01-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer – 45 mL/vial of buffered protein based solution with preservatives	DB01	1 vial
HRP Diluent Solution – 12 mL/vial of buffered protein based solution with preservatives	DB08C	1 vial
Wash Buffer – 50 mL/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution – 11 mL/vial of TMB substrate solution	TMB01	1 vial
Stop Solution – 11 mL/vial of 0.5M HCL	S-STOP	1 vial

Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20 °C. Do not use kit past expiration date.
Streptavidin- HRP Conjugate 100-fold Concentrate and other components may be stored at 2 - 8°C for up to 10 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (350-400rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product be handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum sample may require 1000-2000 dilution. Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
CXCL7 Standard – Reconstitute standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 mL	8000 pg/mL
# 1	150 µL of stock	450µL	2000 pg/mL
# 2	250µL of 1	250µL	1000 pg/mL
# 3	250µL of 2	250µL	500 pg/mL
# 4	250µL of 3	250µL	250 pg/mL
# 5	250µL of 4	250µL	125 pg/mL
# 6	250µL of 5	250µL	62.5 pg/mL
# 7	250µL of 6	250µL	31.25 pg/mL

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent Solution into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few hours.*

Positive Control - Reconstitute the **Positive Control** with 0.5 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*. *Reconstituted Positive Control CAN NOT BE REUSED*.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic bag containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well.
4. Add 100 µL of **Standard**, **sample**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to 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.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 20-25 minutes at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 3 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a 4-parameter curve fit.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 8000 pg/mL may result in inaccurate, low human CXCL7 levels. Such samples require further external predilution according to expected human CXCL7 values with Dilution Buffer in order to precisely quantify the actual human CXCL7 level.

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant human CXCL7.

SPECIFICITY

This assay recognizes both natural and recombinant human CXCL7. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh CXCL7 control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEIN NAME	CROSS-REACTIVITY
Human CXCL7	100%
Human GRO-α	0
Human IL-8	0
Human MCP-1	0
Human SDF-1α	0
Human SDF-1β	0

TYPICAL DATA

These standard curves (4-parameter) are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED)
BLANK	0 (0.075)
31.25	0.029
62.5	0.069
125	0.109
250	0.219
500	0.559
1000	1.219
2000	2.034
8000	3.409

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20-25 min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 3 min