ACTIVE INTERLEUKINE 32 (IL-32) (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN IL-32 BDNF CONCENTRATIONS IN SERUM OR RECOMBINANT

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	ACTIVE IL-32 (HUMAN) ELISA KIT
Catalog No.	SK00704-01
Lot No.	
Formulation	96 T
Standard range	31 - 2000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Type	Serum, recombinant
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Specificity	Human IL-32
Calibration	Human IL-32 recombinant, (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C for 1 month, see page 2 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human IL-32 ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human IL-32 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human IL-32 and antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human IL-32 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human IL-32. The capture antibody can bind to the IL-32 in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody HRP conjugated against sIL-32 is added to the wells. After the last wash to remove any unbound Antibody HRP conjugate, a substrate solution is added to the wells and color develops in direct proportion to the amount of IL-32 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
sIL-32 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against IL-32.	**704-01-01**	**1 plate**
IL-32 Standard – refer to lot vial of recombinant IL-32 in a buffered protein base with preservative; lyophilized.	**704-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot vial, 100-fold concentrated of antibody HRP conjugate against IL-32 with preservative; liquid.	**704-01-03**	**1 vial**
Positive Control- one vial of recombinant IL-32; lyophilized.	**704-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 100 µl/vial, 150-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody & HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB108A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum do not require dilution. The levels of IL-32 in serum samples was very low. For ten research samples were detect by this elisa kit. The data indicated only 4 samples can be detected by this elisa kit at around 900 pg/mL. The other six samples were under the lowest standard 31 pg/mL.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

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 1x Wash Buffer.

IL-32 Standard - Reconstitute the IL-32 standard with refer to lot of Dilution Buffer. 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 the tube #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 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	Refer to lot	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml

Positive Control -Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Pipette 9.395 mL of **Antibody & HRP Diluent solution (DB108A)** into a 15 ml centrifuge tube and transfer 0.105 mL of 100-fold concentrated stock solution to prepare working solution.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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.

5. Add 100 μL of Detection Antibody HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 1 hour on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
8. 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.
9. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human IL-32 (HEK293)	100
Human IL-33	0
Human IL-6	0

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
31.25	0.041
62.5	0.088
125	0.179
250	0.384
500	0.742
1000	1.528
2000	2.235

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 μl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 μl Detection Antibody HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 μl Substrate solution to each well. Incubate 7refer to lot on the plate shaker at RT. **Protect from light**.
↓
Add 100 μl Stop Solution to each well. Read at 450 nm within 5 min.