SOLUBLE AMINOPEPTIDASE P (APP)/XPNPEP2 (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE APP CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL 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	**SOLUBLE APP/XPNPEP2 (HUMAN) ELISA KIT**
Catalog No.	SK00517-01
Lot No.	20114818
Formulation	96 T
Standard range	12.5 - 800 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µL of diluted samples
Sample Type	Serum, EDTA Plasma
Dilution Factor	***80-160 (Optimal dilutions should be determined by each laboratory for each application)***
Specificity	Human soluble APP
Calibration	Human APP recombinant, (HEK293)
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 6 months, see page 2 for more information
This kit contains sufficient materials to run approximately 35~40 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

This Human Soluble Aminopeptidase P (APP)/XPNPEP2 ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human APP from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human APP derived from HEK293 cells animal free cultures and monoclonal antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human APP samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human soluble APP. The capture antibody can bind to the soluble APP in the standard and samples. After washing the plate of any unbound substances, an HRP conjugated monoclonal antibody against sAPP is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of sAPP 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
APP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against soluble APP.	**517-01-01**	**1 plate**
Soluble APP Standard – 800 pg/vial of recombinant soluble APP in a buffered protein base with preservative; lyophilized.	**517-01-02**	**1 vial**
Detection Antibody HRP Concentrate – 105 µL/vial, 100-fold concentrated of monoclonal antibody HRP conjugated against APP with preservative; lyophilized.	**517-01-03**	**1 vial**
Positive Control- one vial of recombinant APP; lyophilized.	**146-01-04**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Wash Buffer - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB03**	**1 bottle**
Stop Solution - 11 mL of 0.25M 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 8 months. For longer storage up to 12 months, unopened Standard, Positive Control, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Detection Antibody -HRP Conjugate Concentrate 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 (200 – 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.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require 80 ~160 fold dilution. A pretest is needed to optimize the dilution of samples. A 40 fold dilution is 5 µL of samples + 195 µl of Dilution Buffer. A 80 fold dilution is 50 µL per well of 40-fold diluted samples + 50 µl per well of Dilution Buffer. A 160 fold dilution is 25 µL per well of 40-fold diluted samples + 75 µl per well of Dilution Buffer.

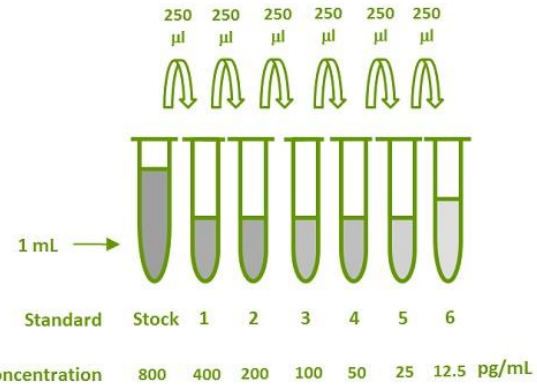
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 25 mL of 20-fold concentrated Wash Buffer Concentrate into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.**

Soluble APP Standard - Reconstitute the soluble APP standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 800 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 the tube #1 to #6. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **800 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution at – 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	800 pg/ml
# 1	250 µl of stock	250 µl	400 pg/ml
# 2	250 µl of 1	250 µl	200 pg/ml
# 3	250 µl of 2	250 µl	100 pg/ml
# 4	250 µl of 3	250 µl	50 pg/ml
# 5	250 µl of 4	250 µl	25 pg/ml
# 6	250 µl of 5	250 µl	12.5 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer to prepare the 10-fold concentrated stock solution. Pipette 0.45 mL of Dilution Buffer and transfer 50 µl of 10-fold concentrated stock solution to prepare working solution. Discard the 1x positive control after use.

Detection Antibody HRP Conjugate- For 96 wells test, freshly pipette 10.395 mL of **Dilution Buffer DB10** into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution. For partial strip test, freshly prepare 900 µL per well of the working solution. Always store the 100-fold concentrated stock solution at 2 ~ 8 °C for 10 months. The 1x working solution should be used in 10~20 min.

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.
9. Add 100 µL of Substrate Solution to each well. Incubate for 15 minutes on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 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 or 4-parameter 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 Soluble APP (HEK293)	100
Human Soluble Neprilysin (HEK293 derived)	0
Human Soluble ACE2 (HEK293)	0

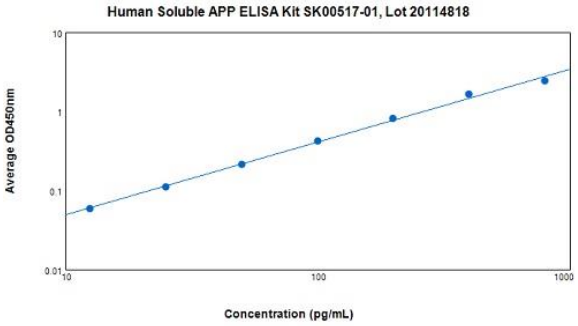
TYPICAL STANDARD CURVE

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

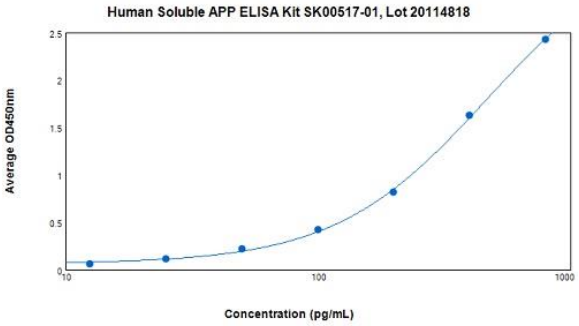
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.058)
12.5	0.059
25	0.111
50	0.214
100	0.417
200	0.813
400	1.622
800	2.429

- Lot No.: 20114818
- Positive Control: 40 ~ 160 pg/mL (log-log)

Standard curve by log-log fit



Standard curve by 4-parameter fit:



The research samples were diluted by Dilution Buffer DB10. Its linearity and recovery was assayed by Human Soluble APP ELISA Kit SK00517-01

Sample	Dilution Factors	Assayed (pg/ml)	Final (ng/ml)	Recovery (%)
Human Serum	40X	837.721	33.508	100
Human Serum	80 X	406.807	32.545	98
Human Serum	160 X	214.693	34.351	103
Human EDTA Plasma	40 X	943.635	37.745	100
Human EDTA Plasma	80 X	483.014	38.641	102
Human EDTA Plasma	160 X	231.300	37.008	98

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 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 for 15 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.

Well Position