HIGH SENSITIVITY CYCLOPHILIN A (CYPA) HUMAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CYPA CONCENTRATIONS IN SERUM AND PLASMA

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	HIGH SENSITIVITY CYPA HUMAN ELISA KIT
Catalog No.	SK00558-06
Lot No.	
Formulation	96 T
Standard Range	50 – 3200 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL per well
Sample Type	Serum, EDTA Plasma
Specificity	Human CYPA
Calibration	Human CYPA Recombinant
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Intra-assay Precision	6 - 8%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 2 months. See page 2 ~3 for detail
This kit contains sufficient materials to run 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High Sensitivity Cyclophilin A (CYPA) Human ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CYPA from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CYPA and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CYPA in samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human CYPA. The capture antibody can bind to the human CYPA in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human CYPA is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. 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 human CYPA immunoreactivity bound in the standard dilutions 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.
_Each laboratory must determine the optimal dilution factors for 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
CYPA CT Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human CYPA.	**558-06-01**	**1 plate**
Human CYPA Standard – 6.4 ng /vial of human CYPA in a buffered protein base with preservative; lyophilized.	**558-06-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated IgG against human CYPA with preservative; lyophilized.	**558-06-03**	**1 vial**
Positive Control - one vial of CYPA; lyophilized.	**558-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate.	**SAHRP**	**1 vial**
Dilution Buffer Concentrate - 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB02**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08B**	**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.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 2 months. For longer storage for up to 10 months, unopened

Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer Concentrate , Antibody Diluent Solution and 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 (350 – 400 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) 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.

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

SAMPLE PREPARATION

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 Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Human CYPA Standard - Reconstitute the CYPA standard with 1 mL 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 tubes #1 to #7. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **3200 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	6400 pg/mL
# 1	250 µl of stock	250 µl	3200 pg/mL
# 2	250 µl of 1	250 µl	1600 pg/ml
# 3	250 µl of 2	250 µl	800 pg/ml
# 4	250 µl of 3	250 µl	400 pg/ml
# 5	250 µl of 4	250 µl	200 pg/ml
# 6	250 µl of 5	250 µl	100 pg/ml
# 7	250 µl of 6	250 µl	50 pg/ml

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

Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB02)** to produce a 10-fold concentrated stock solution. For the 96 wells test, freshly Pipette 9.45 mL of **Antibody Diluent Solution (DB02)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For the partial strip test, freshly prepare 900 ul per strip of working solution. Store the stock solution at -20 °C for a few days.

Streptavidin HRP Conjugate – For the 96 wells test, freshly Pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. The working solution of Streptavidin HRP Conjugate should be used within 10-20 min. For the partial strip test, freshly prepare 900 ul per strip of working solution. Store the stock solution (100-fold concentrated) at 2 ~ 8 °C for 10 months.

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, 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 working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP working solution to each well. Cover with plate sealer. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate Solution to each well. Incubate for 10 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 within 3 minutes, using a microplate reader set to 450 nm.

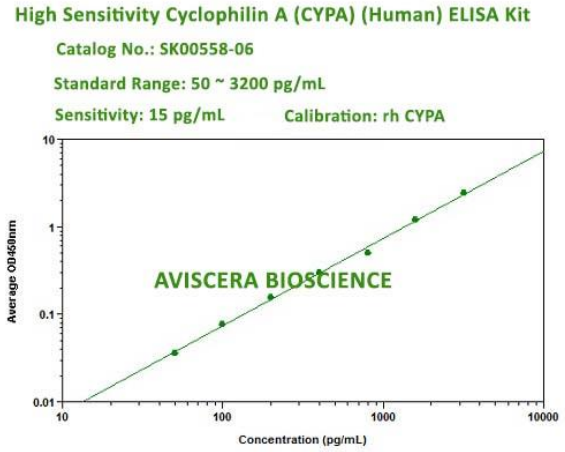
CALCULATION OF RESULTS

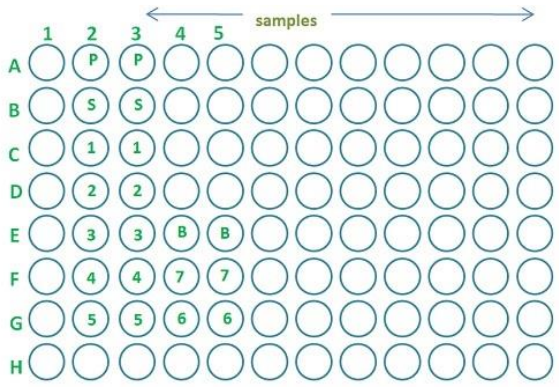
Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit 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.

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)	CORRECTED (AVERAGE OD450NM)
Blank	0 (0.094)
50	0.036
100	0.072
200	0.139
400	0.274
800	0.495
1600	1.109
3200	2.456





SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human CYPA	100
Human CYPB	0
Human CD147 (HEK293)	0

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 of Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Streptavidin-HRP working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl of TMB Substrate Solution to each well. Incubate 10 minutes on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl of Stop Solution to each well. Read 450nm within 3 min.