OriGene Technologies Inc.
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Human TAFI/CPB2 ELISA Kit

Catalog Number:EA102351

Assay Principle

The OriGene Human CPB2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human CPB2 with a 96-well strip plate that is pre-coated with antibody specific for CPB2. The detection antibody is a biotinylated antibody specific for CPB2. The kit contains recombinant Human CPB2 with immunogen: Expression system for standard: NSO; Immunogen sequence: M1-V423. The kit is analytically validated with ready to use reagents.

To measure Human CPB2, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with Washing buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with Washing buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human CPB2 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human CPB2 in the sample.

Overview

Product Name	Human TAFI/CPB2 ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human TAFI/CPB2. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	156pg/ml-10000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	Q96IY4

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Technical Details

Capture/Detection Antibodies	*The capture antibody is a monoclonal antibody from mouse,the detection antibody is polyclonal antibody from goat.*
Specificity	*Natural and recombinant Human CPB2*
Immunogen	*Expression system for standard: NSO; Immunogen sequence: M1-V423*
Cross Reactivity	*There is no detectable cross-reactivity with other relevant proteins.*

Notice Before Application

Please read the following instructions before starting the experiment.

1. *To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.*
2. *Before using the Kit, spin tubes and bring down all components to the bottom of tubes.*
3. *Don't let 96-well plate dry, for dry plate will inactivate active components on plate.*
4. *Don't reuse tips and tubes to avoid cross contamination.*
5. *Avoid using the reagents from different batches together.*

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human CPB2 Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Human CPB2 Standard	2	10ng/tube
Human CPB2 Biotinylated antibody (100x)	1	100 ul
Avidin-Biotin-Peroxidase Complex (100x)	1	100 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Washing buffer(25X)	1	20ml
Plate Sealers	4	Piece

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Required Materials That Are Not Supplied

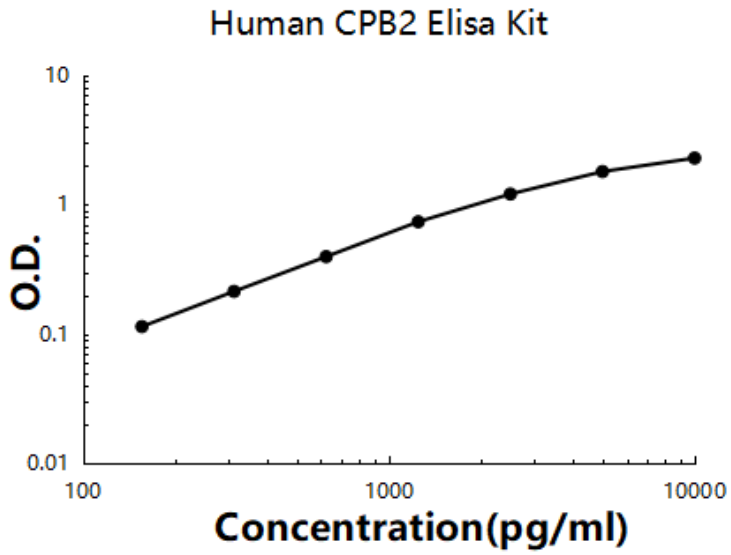
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human TAFI/CPB2 ELISA Kit (EA102351) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0. (TMB reaction incubate at 37°C for 15min)

Concentration(pg/ml)	0.0	156.3	312.5	625	1250	2500	5000	10000
O.D.	0.006	0.114	0.213	0.395	0.734	1.202	1.791	2.275

Human TAFI/CPB2 ELISA Kit standard curve



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

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*

Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

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	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	234	1109	4549	248	1155	4773
Standard deviation	17.08	58.77	209.25	21.32	64.68	229.1
CV(%)	7.3%	5.3%	4.6%	8.6%	5.6%	4.8%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	234	245	212	225	229	12.1	5.2%
Sample 2	1109	1258	1141	1184	1173	55.82	4.7%
Sample 3	4549	4286	4769	4692	4574	184.06	4%

*number of samples for each test n=16.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.
Wash buffer	Prepare 500 ml of working Wash Buffer by diluting the suspended 20 ml Wash Buffer (25 x) with 480 ml of deonized or distilled water.
Biotinylated Anti-Human CPB2 antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human CPB2 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human CPB2 Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Human CPB2 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

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Microplate	*The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.*

Dilution of Human CPB2 Standard

1. *Number tubes 1-8. Final Concentrations to be Tube # 1 –10000pg/ml, #2 –5000pg/ml, #3 – 2500pg/ml, #4 – 1250pg/ml, #5 – 625pg/ml, #6 – 312.5pg/ml, #7 – 156.25pg/ml, #8 – 0.0 (Blank).*
2. *For standard #1, add 1000µl of undiluted standard stock solution to tube #1.*
3. *Add 300 µl of sample diluent to tubes # 2-7.*
4. *To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.*
5. *To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.*
6. *Continue the serial dilution for tube #4-7.*
7. *Tube #8 is a blank standard to be used with every experiment.*

Sample Preparation andStorage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- **Cell culture supernates**: Remove particulates by centrifugation, assay immediately or aliquotand store samples at -20°C.
- **Cell lysate**: Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates atapproximately 10,000 x g for 5 min. Collect the supernatant.Immediately analyze orrefrigerate at -20°C after packaging.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C prior to the experiment (see Preparation Before The Experiment if yo u have missed this information).

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1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 90 min at 37 °C.
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human CPB2 antibody to each well.
7. Cover with plate sealer and incubate for 60 minutes at 37°C.
8. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 30 minutes at 37°C.
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 15-25 minutes at 37° C. (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the rem aining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic- curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on CPB2

Carboxypeptidase B2 (CPB2), also known as carboxypeptidase U (CPU), plasma carboxypeptidase B (pCPB) or thrombin-activatable fibrinolysis inhibitor (TAFI), is an enzyme that, in humans, is encoded by the gene CPB2. CPB2 is synthesized by the liver and circulates in the plasma as a plasminogen-bound zymogen. When it is activated by proteolysis at residue Arg92 by the thrombin/thrombomodulin complex, CPB2 exhibits carboxypeptidase activity. Activated CPB2 reduces fibrinolysis by removing the fibrin C-terminal residues that are important for the binding and activation of plasminogen.