Human Cyclophilin B (CYPB) ELISA Kit Protocol

Protocol for other species is identical except for dilutions of species specific standard. Use the protocol shipped with the kit for your experiment.

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. hCYPB-ELISA
Protocol for other species (mouse and rat Cyclophilin B ELISA kits) is identical except for dilutions of species specific standards.

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Store: 4°C. Expiration: 6 months after arrival.
Safety: Stop solution contains acid. Avoid all contact and inhalation. Wear eye protection.

Human Cyclophilin (CYPB) B ELISA Kit

Part No. hCYPB-ELISA

For research only. Not for diagnostic applications.

Storage: 4°C, six months after arrival

Safety: Stop solution contains acid. Avoid eye and skin contact

Standard Peptide: 1350 pg/ml

Assay Range: 30 pg/ml - 1000 pg/ml

Overview: This ELISA kit is based on the double-antibody sandwich technique to detect soluble human Cyclophilin B. Cyclophilin B (CyPB) is a cyclosporine-binding protein located mainly within the endoplasmic reticulum. It has also been detected in the nucleus where it may maintain a gene regulatory role.

Cyclophilin B has a signal sequence and is therefore associated with the secretory pathway and released in biological fluids. Given it is a soluble protein in the secretory pathway, the primary locations to detect and assay are in biological fluids, such as serum and plasma, cell culture supernatant, and cell and tissue lysates. Use the isolation methods suggested below as a general guide to assay samples of biological fluids and cell culture supernatant. To assay cell or tissue lysate samples, use a non-denaturing lysis buffer to solubilize membranes, and to suspend intracellular Cyclophilin B into a supernatant. The protocol for the FIVEphoton Biochemicals ELISA Lysis and Protein Extraction Buffer (Part No. ELSP-1) provides detailed instructions how to isolate proteins by cell lysis in preparation for ELISA .

Sample Preparation:

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Collect the supernatant to assay. If precipitation appears, centrifuge again. Assay the supernatant fraction.
2. **Plasma:** Use suitable EDTA or heparin as an anticoagulant. Mix for 10-20 min using a stir bar. Centrifuge for 20 min at 2000-3000 rpm. Collect the supernatant to assay. If precipitation appears, centrifuge again, and collect the supernatant to assay.
3. **Urine**: Collect in a sterile container. Centrifuge 20 min at 2000-3000 rpm. Collect the supernatant to assay, If precipitation appears, centrifuge again. Collect the supernatant to assay.
4. **Cell culture supernatant:**. Detection of secretory components: Centrifuge culture media for 20 min at 2000-3000 rpm. Remove and assay the supernatant fraction.
5. **Cell and Tissue Lysates**: Use a non-denaturing detergent buffer containing detergents such as Triton X-100, NP-40 or Tween-20 to lyze to homogenize tissues and lyze cells, dissolve membranes and collect a solubilized supernatant fraction to assay.
6. Samples can be store at -80°C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
7. **Avoid denaturing cell lysis buffers that contain SDS, such as RIPA.**

Experimental Principles

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to determine human Cyclophilin B concentration. Samples are applied to microelisa wells pre-coated with an affinity purified anti-Cyclophilin B capture

antibody. Samples are incubated and then washed. A second goat anti-cyclophilin B-HRP conjugate antibody is then added, followed by incubation and wash. Chromogen solutions A and B are then added resulting in a color change to blue. A stop solution is applied to terminate the reaction, turning the solution to yellow. Absorbance readings at 450 nm corresponding to standard peptide concentrations are used to correlate the concentration of CyPB in the samples.

Table 1. Materials supplied. Store all materials at 4°C

1	Standard peptide: 1350 pg/ml	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent	1.5 ml	8	Chromogen Solution B	6ml
3	Microelisa Strip plate	12 well×8strips	9	Stop Solution	6ml
4	HRP-Conjugate Antibody	6 ml	10	Instruction Manual	1
5	30×wash solution	20ml	11	Sealed bags	1
6	Sample Diluent	6ml	12		

Materials required but not supplied

1. 37°C incubator
2. Standard absorbance microplate reader
3. Precision pipettes and disposable pipette tips
4. De-ionized water
5. Disposable tubes for sample dilution
6. Absorbent paper
7. 96 well dishes for preparation of solutions prior to transfer to the ELISA dish.

Important notes and preparation for the assay

1. It is recommended that the experimenter perform preliminary tests to identify the experimental sample dilution required to meet the assay range. Perform a preliminary assay with samples, using standards at the high and low dilution. Suspend and dilute experimental samples into the “Sample Diluent (Table 1, Component 6)” to meet the assay range, (alternatively, dilute samples in PBS with a protein blocker). A dilution series of several samples may be required to identify the correct concentration that meets the assay range. Concentrate or dilute experimental samples if adjustment is needed. Set aside sufficient experimental sample for reserve to repeat the assay.
2. Determine whether the original sample vehicle inadvertently cross-reacts with the assay by performing vehicle only controls. Furthermore, determine whether ingredients in the vehicle solution inactivate the assay reaction. To identify assay inactivation by the vehicle, dilute the provided standard peptide at ½ concentration of the specified maximum assay range in the vehicle buffer. Then dilute the standard peptide – vehicle solution in Sample Diluent (Component 6) at a dilution 1:5. Now perform the assay reactions. For remedy, dilute experimental samples in the provided “Sample Diluent” (Table 1, Component 6) or prepare samples in another vehicle (such as PBS with protein blocker).
3. The kit should be equilibrated to room temperate for 30 min prior to performing the assay. Store opened microelisa plates in a sealed plastic bag at 4°C. A multi-channel sampler that has been calibrated for accuracy is a preferred method to apply samples. Plates should be sealed during the assay. Wells should not be allowed to dry.
4. Perform dilution of the standards and samples in separate tubes or separate 96-well plates, not in the ELISA wells. Transfer solutions simultaneously to the ELISA dish.
5. It is recommended that samples are assayed in duplicate to address pipetting error.

6. Use new applicator tips and ELISA plate sealants to avoid cross-contamination.
7. Do not mix reagents from other ELISA kits.
8. Note that sodium azide in samples that is not washed away may inhibit HRP.
9. When calculating concentration of your sample, make sure to take into account the dilution factor.
10. If the wash solution crystallizes during storage at 4°C, heat solution at 37°C and shake until crystals suspend.

Assay procedures

Standard and Sample Preparation. Standards and Samples should be added simultaneously to wells. **Prepare the standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Do not prepare samples in the ELISA plate.**

Assay procedure

1. Set aside and mark 12 wells for standard peptide dilutions. Configure six concentrations of standard peptide in duplicate, suspended and mixed as indicated below in a separate 96-well dish. There should be 6 wells in duplicate, therefore 12 wells in total. The final total volume in each well should be 50µl. See Table 2 for standard dilutions.

Table 2. Standard Dilutions

Well	Standard Concentration	Standard Number	Dilution Instructions
1	900 pg/ml	1	Mix 100µl Standard Peptide (Table 1, Component 1) with 50µl Standard Diluent (Table 1, Component 2). Remove 100µl to make standard 3.
2	900 pg/ml	2	Mix 100µl Standard Peptide with 50µl Standard Diluent. Remove 100µl to make standard 4.
3	600 pg/ml	3	Mix 100µl Standard Number 1 with 50µl Standard Diluent. Remove 100µl to make standard 5
4	600 pg/ml	4	Mix 100µl Standard Number 2 with 50µl Standard Diluent. Remove 100µl to make standard 6
5	300 pg/ml	5	Mix 100µl Standard Number 3 with100µl Standard Diluent. Remove 100µl to make standard 7. Remove 50µl, discard.
6	300 pg/ml	6	Mix 100µl Standard Number 4 with100µl Standard Diluent. Remove 100µl to make standard 8. Remove 50µl, discard.
7	150 pg/ml	7	Mix 100µl Standard Number 5 with 100µl Standard Diluent. Remove 100µl to make standard 9. Remove 50µl, discard.
8	150 pg/ml	8	Mix 100µl Standard Number 6 with 100µl Standard Diluent. Remove 100µl to make standard 10. Remove 50µl, discard
9	75 pg/ml	9	Mix 100µl Standard Number 7 with 100µl Standard Diluent. Remove 50µl to make standard 11. Remove 100µl, discard.
10	75 pg/ml	10	Mix 100µl Standard Number 8 with 100µl Standard Diluent. Remove 50µl to make standard 12. Remove 100µl, discard.
11	32.5 pg/ml	11	Mix 50µl Standard Number 9 with 50µl Standard Diluent. Remove 50µl, discard.
12	32.5 pg/ml	12	Mix 50µl Standard Number 10 with 50µl Standard Diluent. Remove 50µl, discard.

2. Set up 2 blank wells separately. In blank wells, add 40µl of the provided Sample Diluent (Component 6) and 10µl of sample vehicle; do not add sample. Perform all other procedures of the assay, except do not add HRP-conjugated antibody to the blank wells.

3. For experimental samples, add 40µl "Sample Diluent," and then add 10µl of experimental sample. Mix the solution. This creates a 5X dilution factor of sample which should be accounted for later when calculating sample concentration.

4. Transfer the standard peptide solutions, blank well solutions and experimental samples simultaneously to the ELISA plate from the 96-well dish used for solution preparation.

5. Use the closure membrane to enclose the plate, mix gently with a rotating table, and incubate for 30 min at 37^{o}C, or 1 hr at RT.

6. Dilute the 30X Wash Solution (Table 1, Component 5) with dH_20. Make 3 ml of 1X wash solution for each assay well.

7. After the first incubation period, discard the liquid in the wells by gently aspirating. Turn the plate upside down and gently pat dry the plate with an absorbent paper. To wash the wells, fill each well with 100 µl of 1X Wash Solution, oscillate gently with the rocker table for 30 sec, and then aspirate off liquid. Pat dry the microplate with absorbent paper. Repeat the wash steps 5 times.

8. Prepare in a separate 96-well dish 50 µl HRP-conjugate reagent (Table 1, Component 4) for each well. Simultaneously transfer 50 µl HRP-conjugate reagent to each well, except for blank wells. Rotate gently for 1 hr at 37^{o}C for 30 min or 1 hr at RT.

9. Wash the wells as described in above 5 times. Remove liquid from the wells.

10. Simultaneously transfer 50 µl of Chromogen Solution A (Table 1, Component 7) and 50µl of Chromogen Solution B (Table 1, Component 8) to each well (prepare these solutions beforehand in a separate 96-well dish). Gently mix for 15 min at 37^{o}C in the dark.

11. Simultaneously add 50 µl of Stop Solution (Table 1, Component 9) to each well. Upon addition of stop solution, the blue color should immediately change to yellow.

12. Measure the optimal density (OD) at 450 nm within 15 minutes of adding the stop solution. Set the blank wells as zero.

13. If sample readings are higher than the most concentrated standard, you may wish to dilute the samples further and assay again.

Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish

to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Remember that samples were diluted 5 fold in the assay in your final calculation. Other methods of data analysis to calculate concentrations of your samples can also be applied.

Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at RT or 30 min at 37^{o}C.

Wash each well five times.

Add HRP conjugate antibody to each well, incubate for 1 hr at RT or 30 min at 37^{o}C.

Wash each well five times

Add chromogen solutions A and B, 15 min at 37^{o}C

Add stop solution

Measure OD 450 nm within 15 min