Human E2/Ubiquitin-conjugating enzyme (E2/UBCE) ELISA Kit – Biotin Detection Antibody Format 96T

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FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. hE2UBCE-ELISA

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Store: 2-8°C. Expiration: 12 months after arrival.
Safety: Stop solution contains acid. Avoid contact and inhalation. Wear eye protection.

Human E2/Ubiquitin-conjugating enzyme (E2/UBCE) ELISA Kit– Biotin Detection Antibody Format

Part No. hE2UBCE-ELISA Biotin

For research only. Not for diagnostic applications.

Storage: 2-8^{o}C, expires 12 months after arrival

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

Standard Peptide: 32 ng/ml

Assay Range: 0.1 ng/ml - 30 ng/ml

Overview: Please read this protocol completely prior to using the product. The kit measures Human E2/UBCE in sandwich ELISA format. The ELISA plate is provided pre-coated with an anti-E2/UBCE monoclonal antibody. The experimenter mixes the samples with a provided polyclonal anti-E2/UBCE antibody labeled with biotin and with the provided streptavidin-HRP solution *prior* to the addition to the ELISA plate. The sample-antibody-biotin-streptavidin-HRP mixture is then added to the ELISA plate, which is incubated for 60 min at 37^{o}C. Unbound biotin-linked antibody is washed away and color reagents are added that change the color of the solution to blue. The blue solution then turns yellow after addition of the stop solution. The absorbance of the resulting yellow solution at 450nm corresponds to the concentration of Human E2/UBCE in the samples.

Sample Preparation - Generic Overview

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Collect supernatant for 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 supernatant. If precipitation appears, centrifuge again and collect the supernatant.
3. **Urine**: Collect in a sterile container. Centrifuge 20-min at 2000-3000 rpm. Collect supernatant, If precipitation appears, centrifuge again. Collect supernatant for assay.
4. **Cell culture supernatant:** Detection of extracellular components: Centrifuge culture media for 20 min at 2000-3000 rpm. Collect supernatant for assay.
5. **Membrane fraction:** Lyse cells with a non-denaturing detergent buffer (such as FIVEphoton Biochemicals part no. ELSP-1). Sediment debris and assay the clarified supernatant fraction.
5. Samples can be store at -80^{o}C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
6. **Avoid denaturing cell lysis buffers that contain SDS such as RIPA buffer.**
7. Samples can be diluted in a sample dilution solution with the formula: 1% BSA in PBS pH 7.4. A 1:5 dilution is recommended. i.e. 10 μl sample, 40 μl sample dilution solution.

Table 1. Materials supplied. Store all materials at 4^{o}C

1	Standard peptide: 32 ng/ml	0.5ml	7	Chromogen Solution A	6 ml
2	Standard diluents (1% BSA in PBS pH 7.4	3 ml	8	Chromogen Solution B	6 ml
3	Anti-E2/UBCE Coated ELISA plate (96T)	12 well×8 strips	9	Stop Solution	6 ml

4	Streptavidin-HRP	6 ml	10	Instruction Manual	1
5	30×wash solution	20 ml	11	Closure Membrane	2
6	Anti E2/UBCE antibody labeled with biotin (detection antibody)	1ml	12	Sealed bags	1

Materials required but not supplied

1. Sample dilution buffer (1% BSA in PBS pH 7.4)
2. 37°C incubator
3. Standard absorbance plate reader
4. Precision pipettes and disposable pipette tips
5. De-ionized water
6. Disposable tubes for sample dilution
7. Absorbent paper

Important notes and preparation for the assay

1. Make a solution that is 1% BSA in PBS pH 7.4 for dilution of samples (i.e." sample dilution solution").
2. Equilibrate the ELISA plate strips to room temperature for at least 30 minutes. The plate strips should be kept in a zip lock bag to prevent drying of the wells. Store unused sections of the ELISA plate in a sealed bag at 4°C.
3. Use new pipettes tips at each step to prevent contamination.
4. Do not use reagents from other ELISA kits..
5. Substrate B is light sensitive. Limit light exposure.
6. **Manual Wash Method:** Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100μl wash solution and repeat 5 times. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells.

Assay procedures

Blanks, standard and sample preparation Prepare the blanks, standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Note that the sample – detection antibody – HRP complex are prepared prior to addition of the samples to wells. Do not prepare samples in the ELISA plate.

Assay procedure

1. Standard dilutions. Use Table 2 as a guide for dilutions of standards in the provided standard diluent solution. Make dilutions in separate tubes or multi-well dishes, not in the ELISA dish. Use 2 replicates for the blank, standard and sample wells.

Table 2. Standard Dilutions

Standard Concentration	Standard Number	Dilution Instructions
16 ng/ml	7	120μl original standard + 120μl standard diluents solution, mix
8 ng/ml	6	120μl standard No. 7 + 120μl standard diluents solution, mix

4 ng/ml	5	120µl standard No. 6 + 120µl standard diluents solution, mix
2 ng/ml	4	120µl standard No. 5 + 120µl standard diluents solution, mix
1 ng/ml	3	120µl standard No. 4 + 120µl standard diluents solution, mix
0.5 ng/ml	2	120µl standard No .3 + 120µl standard diluents solution, mix
0.25 ng/ml	1	120µl standard No. 2 + 120µl standard diluents solution, mix

2. Blank, standard and sample preparation prior to addition to the ELISA wells:

a) Blank wells: Set aside 2 blank wells. Only add chromogen solutions A and B and then stop solution later during the procedural steps while developing the ELISA.

b) Standard solution preparation: Add 50µl standard (that was previously diluted) and 50µl streptavidin-HRP. (Detection antibody labeled with biotin has been added in advance to the standards, therefore detection antibody is not added to prepare the standards solutions).

c) Sample wells: Dilute 10ul sample in 30µl sample diluent solution (1% BSA in PBS pH 7.4 – sample diluent is made by the experimenter), then add 10µl E2/UBCE detection-biotin antibody and 50µl streptavidin-HRP. Mix gently; do not vortex.

d) Transfer the standard and sample solutions simultaneously to the ELISA dish. Seal the wells with a ziplock bag and rotate the plate gently for 60 minutes at 37^{o}C.

3. Preparation of wash solution: Dilute the 30X wash solution to 1X with dH_20. Prepare 600µl 1X wash solution per well.

4. Wash: Carefully remove the seal plate membrane or zip lock bag that protects the wells from drying as not to cross-contaminate liquid. Aspirate off the liquid. Fill each well with 100µl 1X wash solution. Aspirate the liquid after 30 seconds. Then repeat the wash procedure five times. Quickly blot dry the plate.

5. Color development: First add 50µl chromogen solution A to each well and then add 50µl chromogen solution B to each well. Shake gently to mix solutions A and B. Incubate the plate protected from light for 10 minutes at 37^{o}C.

6. Stop solution: Add 50îl Stop Solution to each well to stop the reaction (the blue color changes into yellow).

7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank well as zero, measure the absorbance (OD) of each well at 450nm.

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. Take into account dilution factors in your calculation of E2/UBCE concentration. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

Flow chart of the procedures

Separately prepare standards, blank and samples with detection antibody and HRP-streptavidin as required

Incubate with the ELISA plate for 1 hr at 37^{o}C.

Wash the plate five times.

Add Chromogen solutions A and B. Incubate for 10 min at 37^{o}C for color development.

Add stop solution

Measure OD value at 450nm within 10 min