Cytochrome P450 3A4 (CYP3A4) human ELISA Kit

(Catalog # K7570-100, 100 assays; Storage: refer to Section V)

I. Introduction:
Cytochrome P450 3A4 (CYP3A4) is a monoxygenase anchored to the Endoplasmic reticulum membrane, its expression being highest in liver and intestinal cells. CYP3A4 alone metabolizes ~50% of all drugs on the market. BioVision’s human CYP3A4 ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. A mouse monoclonal human CYP3A4-specific antibody is coated on the 96-well plate. Standards or samples are then added to the appropriate wells with a biotin-conjugated antibody specific to CYP3A4. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubated. After TMB substrate solution is added, only those wells that contain CYP3A4, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color to form a blue product. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change from blue to yellow is measured spectrophotometrically at 450nm. The density of yellow color is proportional to the human CYP3A4 captured onto the plate. The concentration of CYP3A4 in the samples is then determined by comparing the O.D. of the samples to the standard curve. This ELISA kit shows no detectable cross-reactivity with related isoforms. Detection Range: 0.128 ng/ml – 20 ng/ml. Sensitivity: 0.128 ng/ml. Assay Precision: Intra-assay CV<10% and Inter-assay CV<12%.

II. Application:
Quantitative protein detection, measure expression of CYP3A4 after induction, compare CYP3A4 content in healthy versus diseased or drug-treated samples

III. Specificity:
Native and recombinant human CYP3A4

IV. Sample Type:
- Tissue or cell lysates
- Microsomes from human tissues or cells

V. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K7570-100</th>
<th>Part No.</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well plate coated with anti-CYP3A4 Ab</td>
<td>12 strips x 8 wells</td>
<td>K7570-100-1</td>
<td>-20°C</td>
</tr>
<tr>
<td>Human CYP3A4 standard (20 ng/vial), lyophilized</td>
<td>2 vials</td>
<td>K7570-100-2</td>
<td>-20°C</td>
</tr>
<tr>
<td>Detection Reagent A</td>
<td>120 μl</td>
<td>K7570-100-3</td>
<td>-20°C</td>
</tr>
<tr>
<td>Detection Reagent B</td>
<td>120 μl</td>
<td>K7570-100-4</td>
<td>-20°C</td>
</tr>
<tr>
<td>Standard diluent</td>
<td>20 ml</td>
<td>K7570-100-5</td>
<td>4°C</td>
</tr>
<tr>
<td>Wash Buffer (30X concentrate)</td>
<td>20 ml</td>
<td>K7570-100-6</td>
<td>4°C</td>
</tr>
<tr>
<td>Assay diluent A (2X concentrate)</td>
<td>6 ml</td>
<td>K7570-100-7</td>
<td>4°C</td>
</tr>
<tr>
<td>Assay diluent B (2X concentrate)</td>
<td>6 ml</td>
<td>K7570-100-8</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB</td>
<td>9 ml</td>
<td>K7570-100-9</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop solution</td>
<td>6 ml</td>
<td>K7570-100-10</td>
<td>4°C</td>
</tr>
<tr>
<td>Plate Sealers</td>
<td>4</td>
<td>K7570-100-11</td>
<td>RT</td>
</tr>
</tbody>
</table>

VI. User Supplied Reagents and Equipment:
- Microplate reader capable of measuring absorbance at 450 nm +/- 10 nm.
- Absorbent paper.
- Eppendorf tubes.
- Deionized or distilled water.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended.

VII. Storage Conditions and Reagent Preparation:

Can be stored at the appropriate temperatures for use within 6 months if stored unopened. Best when used within 1 month after first thaw. We recommend storing individual components at the recommended temperatures. Avoid repeated freeze-thaw cycles. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Return any unused wells to the pouch containing desiccant, reseal along entire edge and store at -20°C. Bring all kit components and samples to room temperature (18-25°C) before starting. Protect the TMB solution from light.

Reconstitution of the human CYP3A4 standard: Two vials of CYP3A4 standard (20 ng/vial) are included in each kit. Use one vial for each experiment. Prepare 20 ng/ml of humanCYP3A4 standard solution by adding 1 ml of standard diluent into one of the vials, shake gently (do not foam). Label 7 Eppendorf tubes with 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml and 0 ng/ml respectively. Aliquot 0.5 ml of the standard diluent into each tube. Add 0.5 ml of the 20 ng/ml CYP3A4 standard solution into 1st tube and mix to make 10 ng/ml. Transfer 0.5 ml from this tube to 2nd tube and mix to make 5 ng/ml. Transfer 0.5 ml from this tube to 3rd tube and mix, and so on until 0.3125 ng/ml. The standard solutions are best used within 15 mins.

Preparation of Assay diluent A and Assay diluent B working solutions: Dilute 6 mL of Assay Diluent A or B Concentrate (2X) with 6 mL of deionized or distilled water. Do NOT freeze the working solutions.

Preparation of Detection Reagent A and Detection Reagent B working solutions: Mix gently until any particles are completely dissolved. Centrifuge the stock vials. Dilute Detection Reagent A and Detection Reagent B 1:100 with the Assay diluent A and Assay diluent B working solutions respectively.

Preparation of Wash solution: Dilute 20 mL of Wash Solution concentrate (30X) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1X). Mix without creating foam. If crystals have formed in the Wash concentrate (30X), warm to room temperature and mix gently until the crystals are completely dissolved.
VIII. Sample Preparation and Storage:

Rinse tissues in ice-cold PBS (0.01mol/L pH 7.0-7.2) to remove excess blood thoroughly and weigh before homogenization. Mince the tissues to small pieces and homogenize in PBS with a glass homogenizer on ice. The resulting homogenate can be sonicated in addition to achieve complete lysis. Centrifuge for 10-15 mins. at 5000g. Remove the supernatant and assay immediately or aliquot and store at -20°C or -80°C. Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Primary cells like hepatocytes can be collected after removal of any extracellular matrix. Wash in cold PBS and proceed with lysis preparation by 2-3 cycles of freeze-thaw. Analyze immediately or aliquot and store.

Notes:

a. Store samples to be assayed within 24 hrs. at 2-8°C. For long-term storage, aliquot and freeze at -20°C (≤1 month) or -80°C (≤2 months). Avoid repeated freeze-thaw cycles. Bring samples to room temperature for the assay. Samples with hemolysis are not recommended. Tissue or cell lysates prepared by commercial lysis buffers may cause unexpected ELISA results due to protein denaturation or interference from chemicals.

b. Fresh samples yield the best results. The user needs to estimate the concentration of CYP3A4 in the sample and select a proper amount or dilution factor so that the CYP3A4 concentration used per well falls near the middle of the linear regime of the standard curve.

IX. Assay Protocol:

When diluting samples and reagents, they must be mixed completely and evenly. The 96-well plate should not be dry at any time (drying will inactivate the active components on the plate).

1. Aliquot 0.1 ml per well of the 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml and 0 ng/ml human CYP3A4 standard solutions into the pre-coated 96-well plate. Add 0.1 ml each of the properly diluted human cell/tissue or microsomal samples to each empty well.

Notes:

a. Carefully add samples and reagents without touching the walls of the wells and mix gently without creating foam or bubbles.

b. We recommend that each human CYP3A4 standard solution and each sample is measured in duplicate for best results.

c. We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution. Do not reuse tips and tubes to avoid cross contamination.

2. Seal the plate and incubate at 37°C for 2 hours. Remove the cover, discard the liquid, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.

3. Add 0.1 ml of Detection Reagent A working solution into each well, cover the plate with the plate sealer and incubate the plate at 37°C for 60 min. Remove the cover, discard the liquid and wash plate 3 times. Dispense 350 µl of the 1X wash solution, each time leaving the wash solution in the wells for 1-2 min before gently aspirating the washing buffer (without touching the side walls). After the final wash, decant any remaining liquid and blot the plate onto paper towels or other absorbent material. Automated washers can also be used.

4. Add 0.1 ml of Detection Reagent B working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 1X wash solution as described in step 3. After the final wash, remove the washing buffer and blot the plate onto paper towels or other absorbent material.

5. Add 90µl of prepared TMB solution into each well, cover with a new plate sealer and incubate plate at 37°C in dark for 25-30 min.

Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. Different shades of blue can be seen in the wells proportional to the amount of human CYP3A4 in the standards and samples.

6. Add 50µl stop solution into each well. The color changes into yellow immediately. Mix thoroughly by tapping the sides of the plate without bubble formation.

7. Read absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

8. Calculation: Relative OD_{450} = OD_{450} of each well – OD_{450} of zero standard well. The standard curve can be plotted as the relative O.D. of each standard solution (X) vs. the respective concentration of the standard solution (Y). Creating the standard curve using log-log axes is recommended. The human CYP3A4 concentration in the samples can be interpolated from the standard curve.

Note: if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample.

Figure: Typical CYP3A4 Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

Note: O.D. values of the standard curve may vary from this Fig.

X. RELATED PRODUCTS:

- Cytochrome P450 3A4 (CYP3A4) Mouse ELISA Kit (K7571)
- Cytochrome P450 3A4 (CYP3A4) Activity kit (K701-100)
- Cytochrome P450 2D6 (CYP2D6) Rat ELISA Kit (K7871)
- Cytochrome P450 Reductase (CPR) Activity Kit (Colorimetric) (K700-100)
- Cytochrome P450 Reductase (CPR) Human ELISA Kit (K7871)
- Cytochrome P450 Reductase (CPR) Rat ELISA Kit (K7872)

FOR RESEARCH USE ONLY! Not to be used on humans.