Datasheet for ABIN454180

CYP3A4 ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	CYP3A4
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	78-5000 pg/mL
Minimum Detection Limit:	78 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of human alpha 1-microglobulin, alpha 1-MG concentrations in urine and other biological fluids.
Sample Type:	Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural human alpha 1-MG.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	< 1.56 ng/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.
Characteristics:	Homo sapiens,Human,Cytochrome P450 3A4,Albendazole monooxygenase,Albendazole

Product Details

	sulfoxidase,CYPIIIA3,CYPIIIA4,Cytochrome P450 3A3,Cytochrome P450 HLp,Cytochrome P450 NF-25,Cytochrome
Components:	Reagent (Quantity): Assay plate (1×20ml), Standard (2), Sample Diluent (1×20ml), Assay Diluent A (1×10ml), Assay Diluent B (1×10ml), Detection Reagent A (1×120 µl), Detection Reagent B (1×120 µl), Wash Buffer(25 x concentrate) (1×30ml), Substrate (1×10ml), Stop Solution (1×10ml), Plate sealer for 96 wells (5), Instruction (1)
Material not included:	Luminometer. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	CYP3A4
Alternative Name:	CYP3A4 (CYP3A4 Products)
Background:	The lipocalin family can be subdivided into kernal and outlier sets. The kernal lipocalins form the largest self consistent group, comprising the subfamily of alpha-1-microglobulins. The outlier lipocalins form several smaller distinct subgroups: the OBPs, the von Ebner's gland proteins, alpha-1-acid glycoproteins, tick histamine binding proteins and the nitrophorins. Alpha-1-microglobulin (A1M), also known as protein HC (for Heterogeneous Charge), is a low molecular weight protein component of plasma first discovered in pathological human urine. It is a member of the lipocalin superfamily. Although much is now known of its structure and properties, the function and physiological role of A1M remains unclear, although evidence suggests that it functions in the regulation of the immune system. A1M is known to exist in both a free form and complexed to other macromolecules: immunoglobulin A (IgA) in humans and alpha-1-inhibitor-3 in the human. Free A1M is a monomeric protein composed of one 188 residue polypeptide and contains three cysteines, two of which (residues 75 and 173) form a conserved intra-molecular disulphide link. A1M is glycosylated by three separate carbohydrate chains: two complex carbohydrates are N-linked to asparagines at residues 17 and 96, and the other simple carbohydrate is O-linked to threonine at position 5. 22% of the total molecular mass of the protein is derived from carbohydrate. Free A1M is extremely heterogeneous in charge, and is found tightly associated with a chromophore. This chromophoric group is covalently bound to the free cysteine residue at position 34. It also binds retinol as a major ligand, but this is probably distinct from the its covalent chromophore. The glycosylation is different between species. The principal sites of A1M synthesis are the liver and kidney. Half of all human plasma A1M (about 0.03mg/ml) forms a 1:1 complex with about 5% of plasma immunoglobulin A. The resulting macromolecular complex has a molecular weight of 200000, and a plasma concentration of 0.3mg/ml. It can exhibit both antibody activity and affect many

Target Details

	of the biological actions of free A1M. A1M has many affects on the immune system. It inhibits stimulation of cultured lymphocytes by protein antigens, it can induce cell division of 2 lymphocytes, a mitogenic effect that can either be enhanced or inhibited by the action of other plasma components, it inhibits neutrophil granulocyte migration in vitro, and it inhibits chemotaxis.
Pathways:	Steroid Hormone Biosynthesis

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to alpha 1-MG. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for alpha 1-MG and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain alpha 1-MG, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of alpha 1-MG in the samples is then determined by comparing the O.D. of the samples to the standard curve.
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 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 400 ng/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (400 ng/ml). The Sample Diluent serves as the zero standard (0 ng/ml). ng/mL 400 200 100 50 25 12.5 6.25 0 Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20C. Avoid repeated freeze-thaw cycles. Other biological fluids - Remove particulates by

	centrifugation and assay immediately or aliquot and store samples at -20 C or -80 C . Avoid repeated freeze-thaw cycles. Note: Urine to be used within 7 days may be stored at 2-8 C , otherwise samples must 3 stored at -20 C (≤ 1 months) or -80 C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored 4 at 4 C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. 1. Add 100 µ l of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 C . 2. Remove the liquid of each well, don ' t wash. 3. Add 100 µ l of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µ l) using a squirt bottle, multi-channel pipette, 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 µ l of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 C . 6. Repeat the aspiration/wash as in step 4. 7. Add 90 µ l of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 C . Protect from light. 8. Add 50 µ l of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Determine the optical density of each well at once, using a microplate reader set to 450 nm. Important Note: 1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-

Application Details

	wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. 2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 μ l for once pipetting. 3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. 4. For each step in the procedure, total dispensing time for addition of reagents to the 5 assay plate should not exceed 10 minutes. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the alpha 1-MG concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label.

Handling

	2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.