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## **CYP27B1 ELISA Kit**



**Publications** 



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Quantity:	96 tests
Target:	CYP27B1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	31.25 pg/mL - 2000 pg/mL
Minimum Detection Limit:	31.25 pg/mL
Application:	ELISA
Product Details	
Purpose:	For the quantitative determination of human 25-hydroxyvitamin D-1 alpha hydroxylase,
	mitochondrial (CYP27B1) concentrations in serum, plasma, tissue homogenates, cell lysates.
Sample Type:	Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human CYP27B1. No
	significant cross-reactivity or interference between human CYP27B1 and analogues was
	observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the
	cross-reactivity detection between human CYP27B1 and all the analogues, therefore, cross
	reaction may still exist.
Sensitivity:	7.81 pg/mL

## **Product Details**

## Components:

- Assay plate
- Standard
- HRP-avidin (100 x concentrate)
- Biotin-antibody (100 x concentrate)
- · Sample Diluent
- · HRP-avidin Diluent
- · Biotin-antibody Diluent
- Wash Buffer (25 x concentrate)
- · TMB Substrate
- · Stop Solution
- · Adhesive Strip

# Target Details

Target:	CYP27B1	
Alternative Name:	cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1 Products)	
Background:	Abbreviation: CYP27B1	
	Alias: CP2B, CYP1, CYP1alpha, CYP27B, P450c1, PDDR, VDD1, VDDR, VDDRI, VDR,	
	1alpha(OH)ase 25 hydroxyvitamin D3-1-alpha hydroxylase 25-Hydroxyvitamin D3 1alpha-	
	hydroxylase 25-OHD-1 alpha-hydroxylase 25-hydr	
UniProt:	015528	
Pathways:	Metabolism of Steroid Hormones and Vitamin D	

# **Application Details**

Sample Volume: 100 μL Assay Time: 1 - 4.5 h Plate: Pre-coated				
Assay Time:  1 - 4.5 h  Pre-coated  Protocol:  1. Prepare reagents, samples and standards as instructed. 2. Add 100 µL standard or sample to each well. Incubate 2 hours at 37 °C. 3. Remove the liquid of each well, don't wash. 4. Add 100 µL Biotin-antibody (1x) to each well. Incubate 1 hour at 37 °C. 5. Aspirate and wash 3 times. 6. Add 100 µL HRP-avidin (1x) to each well. Incubate 1 hour at 37 °C 7. Aspirate and wash 5 times.	Application Notes:	Optimal working dilution should be determined by the investigator.		
Plate:  Pre-coated  1. Prepare reagents, samples and standards as instructed. 2. Add 100 µL standard or sample to each well. Incubate 2 hours at 37 °C. 3. Remove the liquid of each well, don't wash. 4. Add 100 µL Biotin-antibody (1x) to each well. Incubate 1 hour at 37 °C. 5. Aspirate and wash 3 times. 6. Add 100 µL HRP-avidin (1x) to each well. Incubate 1 hour at 37 °C. 7. Aspirate and wash 5 times.	Sample Volume:	100 μL		
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7. Aspirate and wash 5 times.		5. Aspirate and wash 3 times.		
·		6. Add 100 μL HRP-avidin (1x) to each well. Incubate 1 hour at 37 °C		
8. Add 90 $\mu$ L of TMB Substrate to each well. Incubate for 15-30 minutes at 37 °C. Protect from		7. Aspirate and wash 5 times.		
		8. Add 90 $\mu L$ of TMB Substrate to each well. Incubate for 15-30 minutes at 37 °C. Protect from		

light.

9. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

#### Reagent Preparation:

- 1. Biotin-antibody (1x) Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of Biotin-antibody + 990  $\mu$ L of Biotin-antibody Diluent.
- 2. HRP-avidin (1x) Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of HRP-avidin + 990  $\mu$ L of HRP-avidin Diluent.
- 3. Wash Buffer (1x) If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
- 4. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (2000 pg/mL). Sample Diluent serves as the zero standard (0 pg/mL).

#### Note:

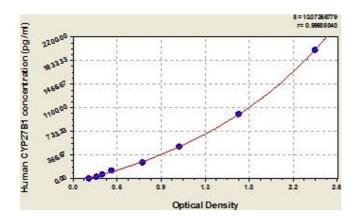
- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- · Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- · Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents.
   Contaminated water or container for reagent preparation will influence the detection result.

#### Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- · Please estimate the concentration of the samples before performing the test. If the values

# **Application Details**

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	are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).
Assay Precision:	Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known
	concentration were tested twenty times on one plate to assess.
	Inter-assay Precision (Precision between assays): CV%<10% Three samples of known
	concentration were tested in twenty assays to assess.
Restrictions:	For Research Use only
Handling	
Storage:	4 °C,-20 °C
Storage Comment:	Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. May be stored for
	up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and
	avoid the damp. Standard May be stored for up to 1 month at 2 - 8° C. If Biotin-antibody don't
	make recent use, better keep it store at HRP-avidin -20°C. Biotin-antibody Diluent Opened kit
	HRP-avidin Diluent Sample May be stored for up to 1 month at 2 - 8°C. Diluent Wash Buffer
	TMB Substrate Stop Solution *Provided this is within the expiration date of the kit.
Expiry Date:	6 months
Publications	
Product cited in:	Liang, Chen, Hu, Yang, Yuan, Wu, Li, Lin, He, Hou, Zhou, Huang: "Protective roles and
	mechanisms of Dendrobium officinal polysaccharides on secondary liver injury in acute colitis.
	in: International journal of biological macromolecules, Vol. 107, Issue Pt B, pp. 2201-2210, (
	2018) (PubMed).



## **ELISA**

Image 1. Typical Standard Curve