# antibodies - online.com







# **Hydroxyproline ELISA Kit**



Image



**Publications** 



( )	11/0	r\ /1	$\triangle 1 $
	$\lor \lor \vdash$	$I \vee I$	ew

Quantity:	96 tests	
Target:	Hydroxyproline (Hyp)	
Reactivity:	Rat	
Method Type:	Competition ELISA	
Detection Range:	0.156 ng/mL - 10 ng/mL	
Minimum Detection Limit:	0.156 ng/mL	
Application:	ELISA	
Product Details		
Purpose:	For the quantitative determination of endogenic rat hydroxyproline (Hyp) concentrations in	
	serum, plasma, tissue homogenates.	
Sample Type:	Plasma, Serum, Tissue Homogenate	
Analytical Method:	Quantitative	
Detection Method:	Colorimetric	
Specificity:	This assay has high sensitivity and excellent specificity for detection of rat Hyp. No significant	
	cross-reactivity or interference between rat Hyp and analogues was observed. Note: Limited by	
	current skills and knowledge, it is impossible for us to complete the cross-reactivity detection	
	between rat Hyp and all the analogues, therefore, cross reaction may still exist.	
Sensitivity:	0.078 ng/mL	
Components:	Assay plate	

- Standard
- HRP-conjugate (100 x concentrate)
- · Sample Diluent
- HRP-conjugate Diluent
- Wash Buffer (25 x concentrate)
- · TMB Substrate
- · Stop Solution
- · Adhesive Strip
- Stop Solution
- · Adhesive Strip

## **Target Details**

Target:	Hydroxyproline (Hyp)	
Alternative Name:	hydroxyproline,Hyp (Hyp Products)	
Target Type:	Amino Acid	
Background:	Нур	
Application Details		
Application Notes:	Optimal working dilution should be determined by the investigator.	

Application Notes:	Optimal working dilution should be determined by the investigator.	
Sample Volume:	50 μL	
Assay Time:	1 - 4.5 h	
Plate:	Pre-coated	
Protocol:	1. Prepare reagents, samples and standards as instructed.	
	2. Set a Blank well without any solution.	
	3. Add 50 µL standard or sample to each well.	
	4. Add 50 μL HRP-conjugate (1x) to each well (Not to Blank well).	
	5. Incubate 1 hour at 37 °C	
	6. Aspirate and wash 5 times.	
	7. Add 90 µL of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from light.	
	8. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.	
Reagent Preparation:	1. HRP-conjugate (1x) - Centrifuge the vial before opening. HRP-conjugate requires a 100-fold	
	dilution. A suggested 100-fold dilution is 10 μL of HRP-conjugate + 990 μL of HRP-conjugate	
	Diluent.	

- 2. 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).
- 3. Standard Centrifuge the standard vial at 6000-10000rpm for 30s before opening. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 10 ng/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 150 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 (10 ng/mL). Sample Diluent serves as the zero standard (0 ng/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:

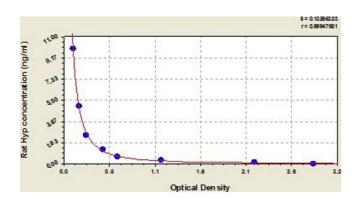
- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles 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 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).

#### Note:

Serum and plasma samples require a 1000-fold dilution into Sample Diluent. The suggested 1000-fold dilution can be achieved by adding 5  $\mu$ L sample to 95  $\mu$ L of Sample Diluent first, then complete the 1000-fold dilution by adding 5  $\mu$ L of this solution to 245  $\mu$ L of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be

# **Application Details**

Application Details		
	determined by users according to their particular experiments. 6	
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 don't make recent use,	
	better keep it store at HRP-conjugate -20°C. Opened kit HRP-conjugate Diluent Sample Diluent	
	May be stored for up to 1 month at 2 - 8°C. Wash Buffer TMB Substrate Stop Solution *Provided	
	this is within the expiration date of the kit.	
Expiry Date:	6 months	
Publications		
Product cited in:	Angbohang, Wu, Charalambous, Eastlake, Lei, Kim, Sun, Limb: "Downregulation of the Canonica	
	WNT Signaling Pathway by TGFβ1 Inhibits Photoreceptor Differentiation of Adult Human Müller	
	Glia with Stem Cell Characteristics." in: <b>Stem cells and development</b> , Vol. 25, Issue 1, pp. 1-12,	
	2016) (PubMed).	



### **ELISA**

Image 1. Typical Standard Curve