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DIO1 ELISA Kit





Publication



Overview	
Quantity:	96 tests
Target:	DIO1
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.15 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.15 ng/mL
Application:	ELISA
Product Details	
Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of DIO1 in rat tissue homogenates, cell lysates.

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of DIO1 in rat tissue homogenates, cell lysates.
Sample Type:	Cell Lysate, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Deiodinase, lodothyronine, Type I (DIO1)
Sensitivity:	0.061 ng/mL
Components:	 Pre-coated, ready to use 96-well strip plate, flat buttom Plate sealer for 96 wells

 Reference Standard · Standard Diluent

- · Detection Reagent A
- · Detection Reagent B
- · Assay Diluent A
- · Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- · TMB Substrate
- · Stop Solution
- Wash Buffer (30 x concentrate)
- · Instruction manual

Target Details

Target:	DIO1
Abstract:	DIO1 Products
Pathways:	Hormone Activity
Application Details	
Comment:	Information on standard material:
	The standard might be recombinant protein or natural protein, that will depend on the specific
	kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin
	300 in the standard as preservative.
	Information on reagents:
	The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash
	solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay
	diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.
	Information on antibodies:
	The provided antibodies and their host vary in different kits.
Sample Volume:	100 μL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards,
	2. Add 100µL standard or sample to each well. Incubate 1 hours at 37 °C,
	3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C,

- 4. Aspirate and wash 3 times,
- 5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
- 6. Aspirate and wash 5 times,
- 7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C,
- 8. Add 50µL Stop Solution. Read at 450nm immediately.

Reagent Preparation:

- 1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit is not used up all at once, remove only the strips and reagents for the current experiment and leave the remaining strips and reagents in the desired condition.
- 2. **Standard** Reconstitute the standard with the Standard Diluent, keep it at room temperature for 10 minutes and shake it gently (do not let it foam). Please prepare tubes with Standard Diluent and make a dilution series. Mix each tube thoroughly before the next transfer. The last tube with Standard Diluent is the blank as 0 mg/mL.
- 3. **Detection Reagent A** and **Detection Reagent B** Spin or centrifuge the stock of Detection Reagent A and B briefly before use. Dilute to working concentration (1:100) with Assay Diluent A or B, respectively.
- 4. **Wash Solution** Dilute 20 mL of Wash Solution Concentrate (30x) with 580 mL of deionized or distilled water to make 600 mL of Wash Solution (1x).
- 5. **TMB Substrate** Aspirate the required amount of solution with sterile tip and do not return the residual solution back into the vial.

Note:

- 1. Serial dilution directly in the wells is not recommended.
- 2. Prepare standard within 15 minutes before assay. Do not dissolve the reagents directly at 37 °C.
- 3. Detection Reagent A and B are sticky solutions, so pipette them slowly to reduce volume errors.
- 4. Reconstitute Standard or working solutions of Detection Reagent A and B carefully according to instructions, avoiding foaming and mixing gently until crystals are completely dissolved. To minimize inaccuracy caused by pipetting, use small volumes and ensure pipettes are calibrated. It is recommended to aspirate more than 10 µL for one-time pipetting.
- 5. The reconstituted Standard, Detection Reagent A and B can only be used once.
- 6. When crystals have formed in the Wash Solution concentrate (30x), warm it to room temperature and mix gently until the crystals are completely dissolved.
- 7. Contaminated water or preparation containers affect 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.

Application Details

- 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).

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

Restrictions:

For Research Use only

Handling

Precaution of Use:

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Storage:

4 °C/-20 °C

Storage Comment:

- 1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C.
- 2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.

Expiry Date:

6 months

Publications

Product cited in:

Wang, Xin, Tang, Li, Zhang, Fan, Miao, Tan, Wang, Zhu: "AMPK Serves as a Therapeutic Target Against Anemia of Inflammation." in: **Antioxidants & redox signaling**, Vol. 27, Issue 5, pp. 251-268, (2018) (PubMed).

Wang, Wu, Cheng, Huang, Zeng, Zhang: "Acidic Polysaccharide from Angelica sinensis Reverses

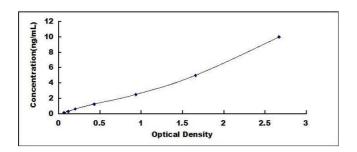
Anemia of Chronic Disease Involving the Suppression of Inflammatory Hepcidin and NF-κB Activation." in: **Oxidative medicine and cellular longevity**, Vol. 2017, pp. 7601592, (2018) (PubMed).

Sal, Yenicesu, Celik, Pasaoglu, Celik, Pasaoglu, Kaya, Kocak, Camurdan, Bideci, Cinaz: "Relationship between obesity and iron deficiency anemia: is there a role of hepcidin?" in: **Hematology (Amsterdam, Netherlands)**, Vol. 23, Issue 8, pp. 542-548, (2018) (PubMed).

Upanan, McKie, Latunde-Dada, Roytrakul, Uthaipibull, Pothacharoen, Kongtawelert, Fucharoen, Srichairatanakool: "Hepcidin suppression in β -thalassemia is associated with the down-regulation of atonal homolog 8." in: **International journal of hematology**, Vol. 106, Issue 2, pp. 196-205, (2017) (PubMed).

Vela, Leshoski, Gjorgievska, Hadzi-Petrushev, Jakupaj, Sopi, Mladenov: "The Role of Insulin Therapy in Correcting Hepcidin Levels in Patients with Type 2 Diabetes Mellitus." in: **Oman medical journal**, Vol. 32, Issue 3, pp. 195-200, (2017) (PubMed).

Images



ELISA

Image 1. Typical standard curve