Version 06-05/24

User's Manual

Human ENTPD5 ELISA Kit

REF **DEIA-XY2208**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of Human ENTPD5 concentration in serum.

General Description

Ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5), also known as CD39 antigen-like 4, ER-UDPase, Guanosine-diphosphatase ENTPD5, Nucleoside diphosphatase Uridine-diphosphatase ENTPD5. This hydrolase is expressed in response to phosphoinositide 3-kinase (PI3K) signaling. Activation of PI3K results in FOXO phosphorylation by AKT1 and loss of ENTPD5 transcriptional repression. It is Up-regulated in PTEN-deficient cells. Uridine diphosphatase (UDPase) that promotes protein N-glycosylation and ATP level regulation.ENTPD5 promotes protein N-glycosylation and folding in the endoplasmic reticulum, as well as elevated ATP consumption in the cytosol via an ATP hydrolysis cycle. Together with CMPK1 and AK1, ENTPD5 constitutes an ATP hydrolysis cycle that converts ATP to AMP and results in a compensatory increase in aerobic glycolysis. ENTPD5 also hydrolyzes GDP and IDP but not any other nucleoside di-, mono- or triphosphates, nor thiamine pyrophosphate. This enzyme Plays a key role in the AKT1-PTEN signaling pathway by promoting glycolysis in proliferating cells in response to phosphoinositide 3-kinase (PI3K) signaling.

Principles of Testing

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human ENTPD5 has been pre-coated onto well plate strips. Standards and samples are added to the wells and Human ENTPD5 present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Human ENTPD5 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Human ENTPD5 bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm.

Reagents And Materials Provided

1. Human ENTPD5 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against Human ENTPD5.
2. Human ENTPD5 Detecion Antibody - 0.2 mg/mL of rabbit mAb antibody against Human ENTPD5 conjugated to horseradish peroxidase (HRP) with preservatives.
3. Human ENTPD5 Standard - Recombinant Human ENTPD5 in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.
4. Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.
5. Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.
6. Color Reagent A - 13 mL of stabilized hydrogen peroxide.

7. Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).
8. Stop Solution - 8 mL of 2 N sulfuric acid.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes and pipette tips
3. Deionized or distilled water
4. Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
5. 500 mL graduated cylinder
6. Tubes for standard dilution
7. Well plate cover or seals

Storage

Unopened Kit:

Store at 2 - 8°C and the kit is stable for 6 months upon receipt.

Opened/Reconstituted Reagents:

Diluted Wash Buffer and Diluted Dilution Buffer - Stored for up to 1 week at 2 - 8°C.

Conjugate, Stop Solution, Unmixed Color Reagent A and Unmixed Color Reagent B - Stored for up to 1 month at 2 - 8°C.

Standard - After reconstitution, store for up to 1 month at -80°C. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.

Microplate Wells - Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal. Stored for up to 1 month at 2 - 8°C.

Specimen Collection And Preparation

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or lower temperature. Avoid repeated freeze -thaw cycles.

Note: The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

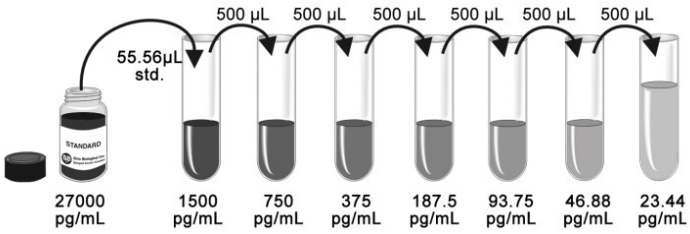
1. Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled

water to prepare 400 mL of Wash Buffer.

2. Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.
3. Detection Antibody - Centrifuge at 10,000 × g for 20 seconds. Dilute to work concentration of 0.5 µg/mL in Dilution Buffer before use.
4. Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.
5. Human ENTPD5 Standard - Reconstitute the Human ENTPD5 Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (Do not turn the vial upside down). Mix the standard to ensure complete reconstitution prior to making dilutions.

   Prepare serially diluted standards as described in the following step: Pipette 1000 µL of Dilution Buffer into the 1500 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 1500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.

   The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove unused microplate strips from the plate frame, return them to the foil pouchcontaining the desiccant pack, and reseal.
3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. Ensure reagent addition is uninterrupted and completed within 15 minutes. Cover/seal the plate and incubate for 2 hours at room temperature.
5. Repeat the aspiration/wash as in Step 3.
6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 20 minutes, using a microplate reader set to 450 nm.

Calculation

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

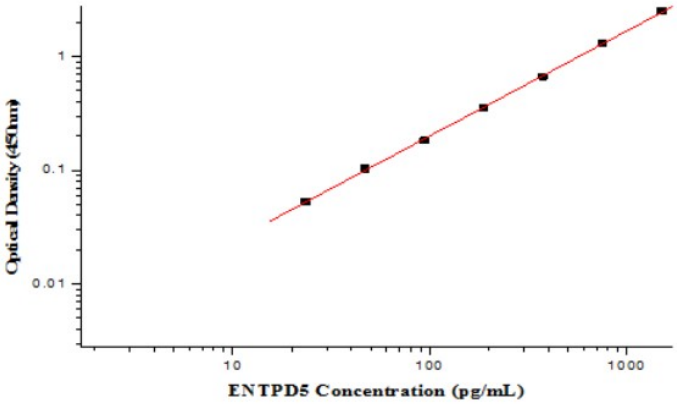
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
23.44	0.053
46.88	0.103
93.75	0.183
187.5	0.354
375	0.668
750	1.296
1500	2.513



Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	5	5	5
Mean (pg/mL)	260	551	1058	244	540	1029
SD	6.13	7.42	26.65	25.15	25.61	44.59
CV (%)	2.4%	1.3%	2.5%	10.3%	4.7%	4.30%

Detection Range

23.44-1500 pg/mL

Sensitivity

The minimum detectable dose (MDD) of Human ENTPD5 is typically less than 5.02 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration

Specificity

This assay recognizes both recombinant and natural Human ENTPD5. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed. A sample containing 5 ng/mL of recombinant mouse ENTPD5 reads as 0.52 ng/mL(10.4% cross-reactivity).

Recombinant human			
CNTF	IL1b	IL3	IL7
LIF	LIFR	TNFb	TGFb1
IL6R	IL1a	IL2	IL4
IL8	IL11	OSM	TNFα
GMCSF	PDGF	ENTPD1	ENTPD2
ENTPD3			

Linearity

		Serum
1:2	recovery of detected	98%
1:4	recovery of detected	105%
1:8	recovery of detected	106%
1:16	recovery of detected	97%

Recovery

The recovery of Human ENTPD5 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Serum (n=3)	94	88 -100%

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.
5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.
6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.
8. Bring all reagents and samples to room temperature before use.
9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.
11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
12. Read the absorbance of each well within 20 minutes after adding the stop solution.