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Thrombopoietin (Human) ELISA Kit

05/19

(Catalog # E4721-100; 96 assay; Storage at 4ºC)

I. Introduction:

TPO is a lineage-specific growth factor, produced in the liver, kidney and skeletal muscle. It stimulates the proliferation and maturation of megakaryocytes and promotes increased circulating levels of platelets in vivo. TPO signals through the c-mpl receptor and acts as an important regulator of circulating platelets. BioVision's ELISA kit is based on the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human TPO. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human TPO and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human TPO, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The concentration of human TPO in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- **Specificity:** This kit recognizes Human TPO in samples. No significant cross-reactivity or interference between Human TPO and analogues was observed.
- **Detection Range:** 31.25—2000 pg/mL
- **Sensitivity:** 18.75 pg/mL

III. Sample Type:

- Plasma, Serum, Other biological fluids

IV. Kit Contents:

Components	E4721-100	Part Number	Storage Temp
Micro ELISA Plate	8 wells x 12 strips	E4721-100-1	-20°C
Standard	2 vials	E4721-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4721-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4721-100-4	-20°C
Standard & Sample Diluent	20 ml	E4721-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4721-100-6	4°C
HRP Conjugate Diluent	14 ml	E4721-100-7	4°C
Wash Buffer (25x)	30 ml	E4721-100-8	4°C
Substrate Reagent	10 ml	E4721-100-9	4°C
Stop Solution	10 ml	E4721-100-10	4°C
Plate Sealer	4	E4721-100-11	RT

V. User Supplied Reagents and Equipment:

- Deionized or distilled water
- Microplate reader with 450 nm wavelength filter

VI. Storage Conditions and Reagent Preparation:

An unopened kit can be stored at 4°C for 1 month. **If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.**

1. HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

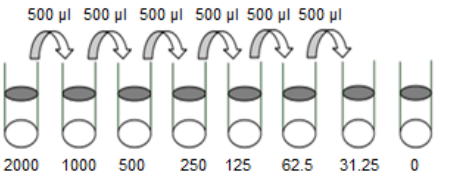
2. Standard preparation: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 1000 pmol/ml stock solution to the first tube and mix up to produce a 500 pmol/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay

3. Biotinylated Detection Antibody: Calculate the required amount before the experiment (50µL/well). Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved

4. Standard preparation: Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for

10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 1000 pmol/ml stock solution to the first tube and mix up to produce a 500 pmol/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.



5. Sample Preparation:

Note: Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles

• **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.

• **Plasma:** Collect plasma using EDTA-Na_2 as anticoagulant. Centrifuge samples for 15 min at 1000× g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

• **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500xg at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

• **Tissue homogenates:** It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.

• **Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000×g at 2~ 8℃. Collect the supernatant for assay.

VII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 100 µl of each **standard or samples** into appropriate wells.

2. Immediately add 100 µl of diluted **Biotinylated Detection Antibody** to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and bubble formation.

3. Aspirate the solution from each well add 350 µl of **1x wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

4. Add 100 µl of diluted **HRP Conjugate** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

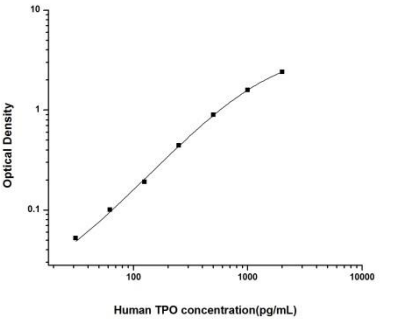
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 3.

6. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution

8. Read the absorbance in micro plate reader set to 450 nm.

9. Calculation: Determine the average of the duplicate readings for each standard and samples. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculated multiplied by the dilution factor. Typical standard curve and data is provided below for reference only.



Conc. (ng/ml)	2000	1000	500	250	125	62.5	31.25	0
OD	2.483	1.66	0.968	0.514	0.264	0.174	0.125	0.073
Corrected OD	2.41	1.587	0.895	0.441	0.191	0.101	0.052	-

VIII. Related Products:

• TPO, rat recombinant (7221) • TPO, human recombinant (4351) • TPO Antibody (5352)

FOR RESEARCH USE ONLY! Not to be used on humans.