cellsciences.com

TPO
Human TPO ELISA Kit

Catalog No: CKH191 **Size**: 1 x 96 wells

Introduction

The Cell Sciences Human TPO ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human TPO in serum, plasma, cell culture supernatants, and urine. This assay employs an antibody specific for human TPO coated on a 96-well plate. Standards and samples are pipetted into the wells and TPO present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human TPO antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TPO bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied with the kit:

Items	Quantity
A. TPO Microplate coated with anti-human TPO	96 wells (12 strips x 8 wells)
B. Wash Buffer Concentrate (20x)	25 ml
C. Recombinant human TPO Standards	2 vials
D. Assay Diluent A for Standard/Sample (serum/plasma) diluent*	30 ml
E. Assay Diluent B (5x) for Standard/Sample (cell culture medium/urine) diluent	15 ml
F. Detection Antibody-Biotinylated anti-human TPO	2 vials (each vial is enough to assay half microplate)
G. Streptavidin-HRP Concentrate (20,000x)	8 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 ml
I. Stop Solution (2 M sulfuric acid)	8 ml

* *Contains 0.09% sodium azide as preservative. Precaution: Sodium azide is a poisonous and hazardous substance which should be handled by trained staff only*.

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Storage of Kit Reagents

Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Note: The whole kit is stable for 1 year when stored at -20°C. Avoid repeated freeze-thaw cycles.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Preparation of Kit Reagents

Bring all reagents and samples to room temperature (18 - 25°C) before use.

Sample Dilution

If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of serum/plasma samples, and Assay Diluent B (Item E) for dilution of culture supernatants and urine.

Assay Diluent B

Dilute 5-fold with deionized or distilled water.

TPO Standard

- Briefly spin the vial of Item C (Recombinant Human TPO Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 100 ng/ml standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 40 µl TPO standard from the vial of Item C, into a tube with 626.7 µl Assay Diluent A or 1x Assay Diluent B to prepare a 6000 pg/ml stock standard solution.
- Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown on next page in Figure 1).
- Mix each tube thoroughly before the next transfer. Gently vortex to mix.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

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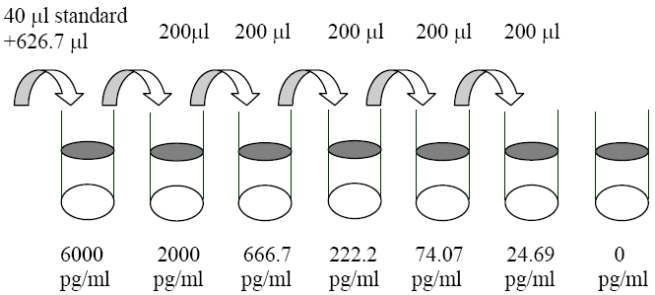
Figure 1



Wash Buffer Concentrate

- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved.
- Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipette up and down to mix gently (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and pipette up and down to mix gently before use.
- Streptavidin-HRP concentrate should be diluted 20,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix thoroughly and then pipette 60 µl prepared 100-fold diluted solution into a tube with 12 ml 1x Assay Diluent B to prepare a final 20,000-fold diluted HRP-Streptavidin solution.

ELISA Method

Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay.

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: TPO Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in Step 3.
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in Step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.
3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

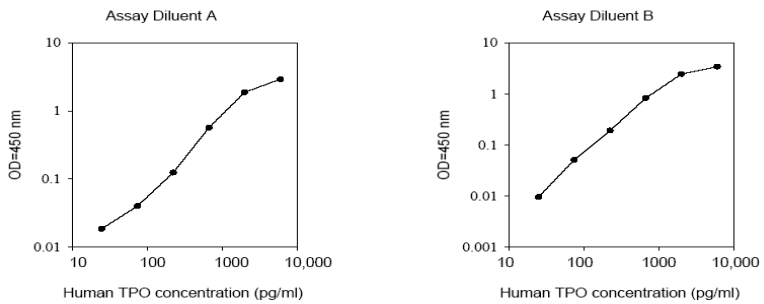
Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Figure 2

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



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Performance and Characteristics

Sensitivity

The minimum detectable dose of TPO is typically less than 25 pg/ml.

Recovery

Recovery was determined by spiking various levels of human TPO into human serum, plasma and cell culture media.

Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	92.21	78-101
Plasma	93.44	80-103
Cell culture media	97.82	84-105

Linearity

Sample Type		**Serum**	**Plasma**	**Cell Culture Media**
1:2	Average % of Expected Range (%)	94 83-103	93 84-102	95 85-103
1:4	Average % of Expected Range (%)	92 84-103	95 85-104	97 82-102
1:8	Average % of Expected Range (%)	95 85-105	94 86-104	101 84-106

Reproducibility

Intra-assay: CV<10%

Inter-assay: CV<12%

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, human BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PDGF, RANTES, SCF, TARC, TGF-β1, TIMP-1, TIMP-2, TNF-α, TNF-β, VEGF).

Troubleshooting Guide

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; ELISA Method Step 2 may change to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage of the ELISA Kit	1. Store your standard at < -20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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