Code No. 27175

Human TPO Assay Kit - IBL

INTRODUCTION

Thrombopoietin (TPO) is a 95 kDa glycoprotein consisting of 332 amino acids that was cloned and identified in 1994. It is produced mainly in the liver and secondarily in the kidneys and bone marrow. It is a hematopoietic factor which stimulates primitive blood cells to grow (proliferate) and evolve into platelet-producing megakaryocytes. TPO is also known as a ligand of c-MPL (Myeloproliferative leukemia protein) and is a key regulator of megakaryocytopoesis and thrombopoiesis.
This ELISA kit can measure human TPO.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of human TPO.

MEASUREMENT RANGE

37.5 - 2,400 pg/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

- This assay kit is capable for the quantitative determination human TPO in cell culture supernatant.
- Human TPO in EDTA-plasma samples are detectable at about 4-fold dilution, however, the recovery rate of 4-fold plasma sample is around 60%.
- In many of serum samples, TPO levels would be less than measurement range.
- This ELISA kit cannot detect *E.coli*-derived recombinant TPO.

KIT COMPONENT

1	Precoated plate : Anti-Human TPO Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human TPO (9B2) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant human TPO	0.5mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody *	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

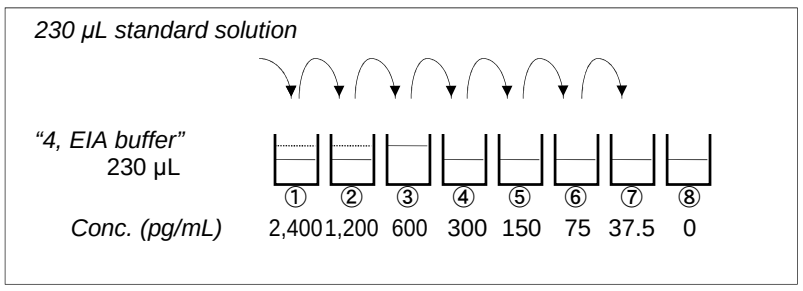
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 4,800 pg/mL humn TPO standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	2,400 pg/mL
Tube-2	1,200 pg/mL
Tube-3	600 pg/mL
Tube-4	300 pg/mL
Tube-5	150 pg/mL
Tube-6	75 pg/mL
Tube-7	37.5 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 2,400 pg/mL and 37.5 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.



5) Dilution of test sample
Test samples should be diluted with "4, EIA buffer" suitably.
If the concentration of human TPO in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation overnight at 4°C with plate lid				
4 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 60 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate overnight at 4°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid. *
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid. *
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

OPERATING PRECATION*

1) Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer" contained in this kit.
3) Duplicate measurement of test samples and standards is recommended.
4) Standard curve should run for each assay.
5) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6) All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7) Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8) Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.

9) Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
10) “6, Chromogen - TMB solution” should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11) After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination. 12) Measurement of O.D. should be done within 30 minutes after addition of “7, Stop solution”.

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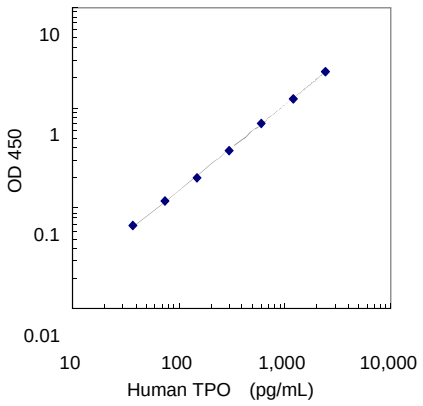
CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

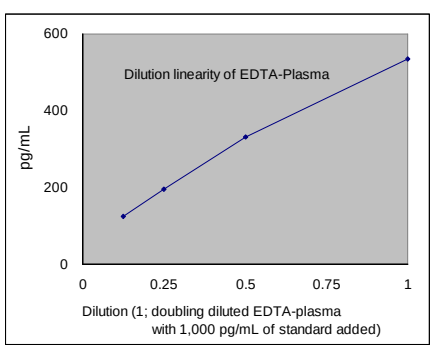
Conc. (pg/mL)	Absorbance (450nm)
2,400	2.383
1,200	1.299
600	0.750
300	0.438
150	0.260
75	0.173
37.5	0.125
0 (Test Sample Blank)	0.057

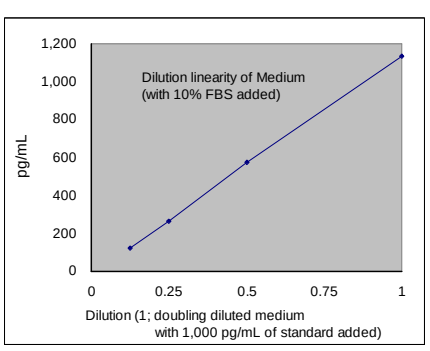


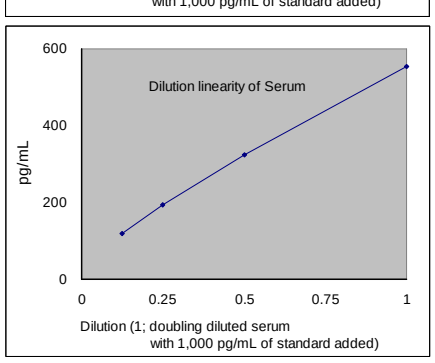
* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Dilution linearity







Specimen	Dilution (x)	Additive Amount (pg/mL)	Theoretical Value (pg/mL)	Measured Value (pg/mL)	%
Medium with 10% FBS	2	1,000	1,000	1,134	113.4
	4	500	500	576	115.2
	8	250	250	265	106.0
	16	125	125	124	99.2
Human Plasma (EDTA)	2	1,000	1,089	535	49.1
	4	500	536	332	61.9
	8	250	265	195	73.6
	16	125	132	125	94.7
Human Serum	2	1,000	1,060	553	55.3
	4	500	518	324	62.5
	8	250	255	193	75.7
	16	125	126	118	93.7

2. Added Recovery Assay

Specimen	Additive Amount (pg/mL)	Theoretical Value (pg/mL)	Measured Value (pg/mL)	%
Medium with 10% FBS (x2)	1,000	1,000	1,118	111.8
	500	500	543	108.6
	250	250	278	111.2
	125	125	131	104.8
Human Plasma (EDTA) (x4)	1,000	1,058	640	60.5
	500	558	343	61.5
	250	308	214	69.5
	125	183	144	78.7
Human Serum (x4)	1,000	1,020	633	62.1
	500	520	317	61.0
	250	270	175	64.8
	125	145	101	69.7

3. Intra - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
1001.00	61.66	6.2	24
428.01	28.81	6.7	24
188.73	14.79	7.8	24

4. Inter - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
992.68	65.58	6.6	7
412.87	37.73	9.1	7
184.85	21.13	11.4	7

5. Sensitivity

17.3 pg/mL
(Calculated by NCCLS method using the standard.) *

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

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Made in Japan.

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