



RES34-EN.01

# **resDetect™ Human TPO ELISA Kit (Residue Testing)** **(Enzyme-Linked Immunosorbent Assay)**

**Catalog Number: RES-A034**

**Pack Size: 96 tests**

**IMPORTANT: Please carefully read this manual before performing your experiment.**

**For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure**

## **INTENDED USE**

Human TPO ELISA Kit (Residue Testing) was developed for the detection and quantitative determination of TPO in human serum and cell culture supernates. It is intended for research use only (RUO).

## **BACKGROUND**

Thrombopoietin (TPO) is a 332 amino acid glycoprotein made primarily in the liver and act as the major physiological regulator of platelet production. TPO binds the TPO receptor, activates JAK and STAT pathways, thus stimulating megakaryocyte growth and platelet production. Additionally, further investigation uncovered that thrombopoietin is a critical cytokine promoting hematopoietic rebound after myeloablation and its transcripts are expressed by multiple cellular sources.

To support the development of CAR-T drugs, ACROBiosystems developed Human TPO ELISA Kit with rigorous methodological validation, which is used evaluation the quality of CAR-T products in drug development and CMC quality control stages. Besides, this kit can also be used for the quantitative determination of GMP human TPO Protein (ACROBiosystems,Cat#GMP-THNH25) concentrations.

## **PRINCIPLE OF THE ASSAY**

This assay kit is used to measure the levels of human TPO by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-TPO Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-TPO Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human TPO bound.

## **PRECAUTIONS**

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.

3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

## **MATERIALS PROVIDED**

**Table1. Materials provided**

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES034-C01	Pre-coated Anti-TPO Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES034-C02	Human TPO Standard	20 µg	Powder	2-8°C	-70°C
RES034-C03	Biotin-Anti-TPO Antibody	20 µg	Powder	2-8°C	-70°C
RES034-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES034-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES034-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES034-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES034-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

*Note: It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

## **SRORAGE**

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

*Note: a. Do not use reagents past their expiration date.*

*b. Find the expiration date on the outside packaging.*

## **REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED**

Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;

37°C Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

## **REAGENT PREPARATION**

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 µg.

*Note: Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

**Table 2. Preparation method**

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES034-C02	Human TPO Standard	20 µg	100 µg/mL	200 µL
RES034-C03	Biotin-Anti-TPO Antibody	20 µg	200 µg/mL	100 µL

## **RECOMMENDED SAMPLE PREPARATION**

### **1. Working Solution Preparation**

#### 1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

#### 1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

#### 1.3 Preparation of Biotin-Anti-TPO Antibody working fluid:

Dilute Biotin-Anti-TPO Antibody to 0.2 µg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

#### 1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

#### 1.5 Sample preparation





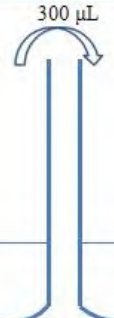
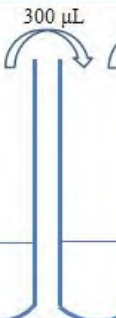



a. If the sample to be tested is the serum, dilute test sample at 1:10 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:9.

b. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

### **2. Preparation of Standard curve**

The concentration of the reconstituted human TPO Calibrator (RES034-C02) is 100 µg/mL, prepare (Std.-0) by diluting 10 µL the reconstituted human TPO Calibrator into 990 µL Sample Dilution Buffer, mix gently well. Then prepare Std.-1 by diluting 10 µL Std.-0 into 490 µL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (400 pg/mL), by diluting 12 µL Std.-1

into 588  $\mu\text{L}$  Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300  $\mu\text{L}$  of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

Tubes/ Solution Code	TPO Standard stock solution	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Operating									
Solution Con.	100 $\mu\text{g}/\text{mL}$	1000 ng/mL	20 ng/mL	400 pg/mL	200 pg/mL	100 pg/mL	50 pg/mL	25 pg/mL	12.5 pg/mL
Dilution Buffer Vol.		990 $\mu\text{L}$	490 $\mu\text{L}$	588 $\mu\text{L}$	300 $\mu\text{L}$	300 $\mu\text{L}$	300 $\mu\text{L}$	300 $\mu\text{L}$	300 $\mu\text{L}$

### 3. Add Samples

Add 100  $\mu\text{L}$  Calibrator and samples to each well. For blank Control wells, please add 100  $\mu\text{L}$  Dilution Buffer.

*Note: It is recommended to set double holes for samples and standard curves to be tested.*

### 4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 5. Washing

Remove the remaining solution by aspiration, add 300  $\mu\text{L}$  of 1 $\times$ Washing Buffer to each well, soak for 10 s, remove any remaining 1 $\times$ Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

### 6. Add Biotin-Anti-TPO Antibody

For all wells, add 100  $\mu\text{L}$  Biotin-Anti-TPO Antibody (dilute to 0.2  $\mu\text{g}/\text{mL}$ ) working solution. Please prepare it for one-time use only.

### 7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### **8. Washing**

Repeat step 5.

### **9. Add Streptavidin-HRP**

For all wells, add 100  $\mu$ L Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

### **10. Incubation**

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### **11. Washing**

Repeat step 5.

### **12. Substrate Reaction**

Add 100  $\mu$ L Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

### **13. Termination**

Add 50  $\mu$ L Stop Solution to each well and tap the plate gently to allow thorough mixing.

*Note: The color in the wells should change from blue to yellow.*

### **14. Data Recording**

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

*Note: To reduce the background noise, subtract the value read at  $OD_{450nm}$  with the value read at  $OD_{630nm}$ .*

## **CALCULATION OF RESULTS**

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve:  $R^2 \geq 0.9900$ .

4. Detection range: 12.5 pg/mL-400 pg/mL. If the OD value of the sample to be tested is higher than 400 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 12.5 pg/mL, the sample should be reported.

**QUICK GUID**

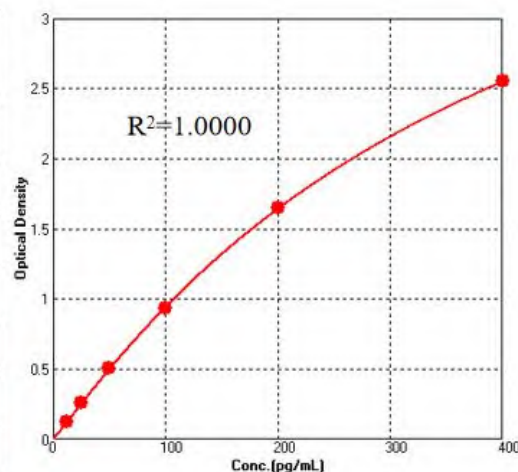




## TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
400	2.618	2.607	2.613	2.550
200	1.697	1.731	1.714	1.652
100	0.991	1.008	1.000	0.937
50	0.580	0.566	0.573	0.511
25	0.327	0.315	0.321	0.259
12.5	0.191	0.185	0.188	0.126
0	0.063	0.062	0.062	/



## SENSITIVITY

The minimum detectable concentration of TPO is 4.817 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## PRECISION

### 1. Intra-assay Precision

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

### 2. Inter-assay Precision

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

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	316.060	72.438	30.941	303.279	71.907	31.401

SD	15.034	3.911	1.583	12.661	1.235	0.519
CV (%)	4.8	5.4	5.1	4.2	1.7	1.7

*Note: The example data is for reference only.*

## **RECOVERY**

Five parts of blank T cell culture supernatant were added with different concentrations of human TPO, and the T cell culture supernatant without human TPO was used as background to calculate the recovery rate. The range of the recovery rate is 94.7-108.9%, and the average recovery is 102.1%.

Sample Type	Average % Recovery	Range(%)
T cell culture supernatant (n=5)	102.1	94.7-108.9

## **LINEARITY**

To assess the linearity of the assay, samples spiked with high concentrations of TPO were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)	Serum
1:2	Average Recovery (%)	100.2	113.3	116.1
	Range (%)	95.6-105.2	107.6-119.6	114.4-118.0
1:4	Average Recovery (%)	99.7	112.9	107.9
	Range (%)	96.6-101.9	110.1-118.2	99.7-114.3
1:8	Average Recovery (%)	100.1	108.7	112.6
	Range (%)	97.1-102.0	98.6-117.5	106.6-117.8
1:16	Average Recovery (%)	95.4	111.7	109.8
	Range (%)	86.6-103.8	101.2-117.4	106.8-116.2

*Note: The example data is for reference only.*

## **SPECIFICITY**

This assay recognizes natural and recombinant human TPO. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

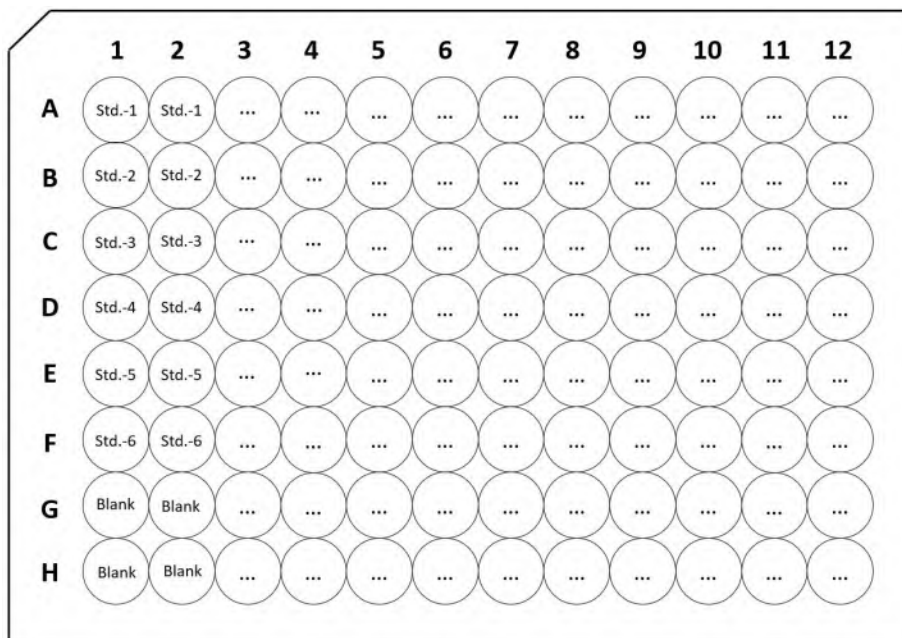
Human			
IL-2	IL-10	GM-CSF	Anti-CD3
IL-3	IL-11	G-CSF	Anti-CD28
IL-4	IL-12B	M-CSF	Anti-CD137
IL-5	IL-15	IFN-alpha 1	FGF basic
IL-6	IL-17A	IFN-gamma	Flt-3 Ligand
IL-7	IL-18	TGF-beta 1	L1R
IL-8	VEGF165	SCF	BMP-2
TNF-alpha			

### **INTERFERING SUBSTANCES**

Verify potential matrix effects by adding different levels of DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
DMSO	2%
HSA	5%

### **PLATE LAYOUT**



*Note: Blank is a Blank Dilution Buffer hole.*

### **TROUBLESHOOTING GUIDE**

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
<b>Poor standard curve</b>	* Inaccurate pipetting	* Check pipettes
<b>Large CV</b>	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
<b>High background</b>	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
<b>Very low readings across the plate</b>	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
<b>Samples are reading too high, but standard curve looks fine</b>	* Samples contain cytokine levels above assay range	* Dilute samples and run again

<p><b>Drift</b></p>	<ul style="list-style-type: none"> <li>* Interrupted assay set-up</li> <li>* Reagents not at room temperature</li> </ul>	<ul style="list-style-type: none"> <li>* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay</li> <li>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts</li> </ul>
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