ClinMax™ Human EPO ELISA Kit, PRO

Catalog Number：CEA-C027

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

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INTENDED USE

This kit is specifically designed for the accurate quantitation of human Erythropoietin (EPO) from cell culture supernates, serum and plasma. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Erythropoietin (EPO) is a glycoprotein cytokine secreted mainly by the kidneys in response to cellular hypoxia; it stimulates red blood cell production (erythropoiesis) in the bone marrow. Low levels of EPO (around 10 mU/mL) are constantly secreted in sufficient quantities to compensate for normal red blood cell turnover. Common causes of cellular hypoxia resulting in elevated levels of EPO (up to 10,000 mU/mL) include any anemia, and hypoxemia due to chronic lung disease.

Erythropoietin is produced by interstitial fibroblasts in the kidney in close association with the peritubular capillary and proximal convoluted tubule. It is also produced in perisinusoidal cells in the liver. Liver production predominates in the fetal and perinatal period; renal production predominates in adulthood. It is homologous with thrombopoietin.

This assay kit is used to measure the concentration of human Erythropoietin (EPO) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti- EPO Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, next add detection antibody Biotin-Anti-EPO Antibody to the plate, incubate and wash the wells. After wash add HRP-Streptavidin to the plate, incubate and wash the wells. Lastly load the substrate into the wells and color develops in proportion to the amount of Erythropoietin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm and 630nm.

LIMITATIONS OF THE PROCEDURE

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

MATERIALS PROVIDED

Table 1. The materials provided in this kit

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CEA027-C01	Pre-coated Anti-EPO Antibody Microplate	1 plate	Solid	2-8 °C	2-8 °C
CEA027-C02	Human EPO Standard	3392IU×2	Lyophilized powder	2-8 °C	-70 °C
CEA027-C03	Biotin-Anti-EPO Antibody Con. Solution	100 µL	Liquid	2-8 °C	2-8 °C
CEA027-C04	Biotin-Antibody Dilution Buffer	8 mL	Liquid	2-8 °C	2-8 °C
CEA027-C05	Streptavidin-HRP Con. Solution	500 µL	Liquid	2-8 °C	2-8 °C
CEA027-C06	HRP Dilution Buffer	15 mL	Liquid	2-8 °C	2-8 °C
CEA027-C07	20× Washing Buffer	50 mL	Liquid	2-30 °C	2-30 °C
CEA027-C08	Sample Dilution Buffer	15 mL ×2	Liquid	2-8 °C	2-8 °C
CEA027-C09	Substrate Solution	12 mL	Liquid	2-8 °C	2-8 °C
CEA027-C10	Stop Solution	6 mL	Liquid	2-30 °C	2-30 °C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge；

10 µL, 200 µL and 1000 µL precision pipettes；

10 µL, 200 µL and 1000 µL pipette tips；

Multichannel pipettes；

Tubes；

Graduated cylinder to prepare Wash Solution；

Deionized water / ultrapure water / distilled water to dilute 20× Washing Buffer.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g within 30 minutes of collection. Assay immediately.

Storage - Samples which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower and will be stable for up to six months. Note: Reduce the number of freeze-thaw cycles.

KIT STORAGE AND EXPIRATION DATE

The unopened kit is stable for 24 months from the date of manufacture if stored at 2°C to 8°C.

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.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (18-25 °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. The freeze-thaw cycle should not exceed 1 time, and the size of the aliquot should not be less than 1696 IU.

Table 2. Preparation of Standard

Catalog	Components	Size (96 tests)	Storage solution concentration	Reconstituted water volume
CEA027-C02	Human EPO Standard	3392 IU	3392 IU /mL	1 mL

RECOMMENDED SAMPLEPREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL 20×Washing Buffer with deionized water/ultrapure water/distilled water to 1000 mL.

1.2 Preparation of Biotin-Anti-EPO Antibody Solution

Prepare Biotin-Anti-EPO Antibody Solution by adding 60 µL of Biotin-Anti-EPO Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

1.3 Preparation of EPO Streptavidin-HRP Solution

Prepare EPO Streptavidin-HRP Solution by adding 240 µL of EPO Streptavidin-HRP Con. Solution to 12 mL of HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

2. Preparation of Calibration curve

The concentration of the reconstituted human EPO Standard（CEA027-C02）is 3392 IU/mL，prepare Cm by adding 10 µL of the reconstituted human EPO Standard to 990 µL Sample Dilution Buffer, gently mix well. Label 6 tubes, one for each standard point: C1, C2, C3, C4, C5, C6. According to the following dilution scheme: Add 15 µL of EPO Cm and 2385 µL of Sample Dilution Buffer to tube C1, thoroughly mix (C1 =212 mIU/mL). Prepare 1:2 serial dilutions for the standard curve as follows: Add 1000 µL of Sample Dilution Buffer into each tube (C2, C3, C4, C5, C6). Transfer 500 µL of liquid from C1 to the tube C2, and thoroughly mix (C2 = 70.67 mIU/mL). Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube C6. Sample Dilution Buffer serves as blank.

3. Add Samples and Biotin-Antibody Solution

Add 50 µL of EPO Standard or samples pre well, then add 50 µL Biotin-Anti-EPO Antibody Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 **℃**) for **2 hours**.

4. Washing

Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 minute. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.

5. Add EPO Streptavidin-HRP Solution

Add 100 µL of EPO Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light**.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.

8. Termination

Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.

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

9. Data Recording

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

Note*: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

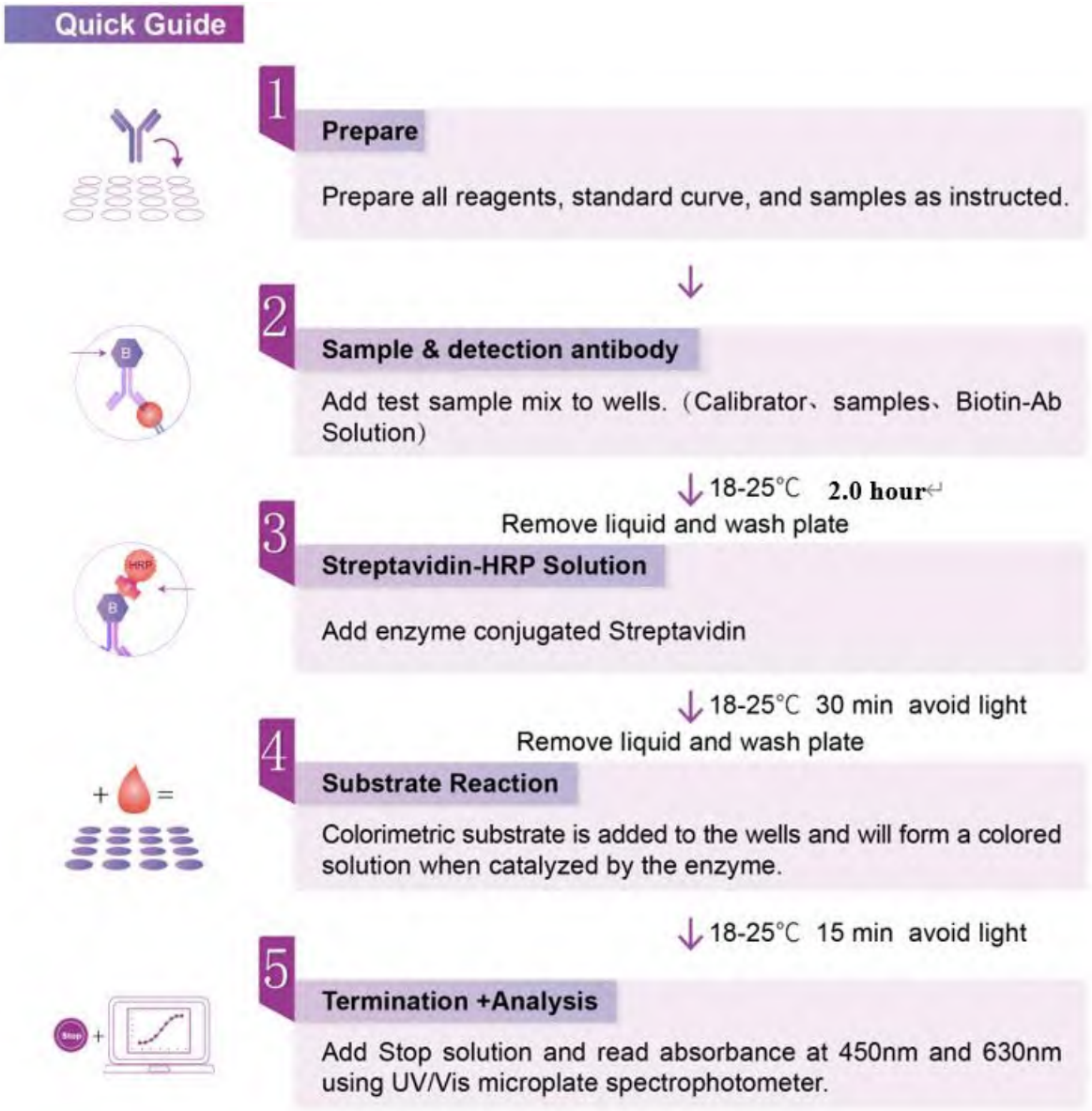
CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Logit-4P regression equation are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS FOR USE

1. All chemicals should be considered as potentially hazardous. It is recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
2. Do not use kit reagents beyond expiration date on label.
3. In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
4. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
5. Glass-distilled water or deionized water must be used for reagent preparation.
6. This kit should be used according to the provided instructions.
7. Do not mix reagents from different lots.
8. Bring all reagents and samples to room temperature (18-25 °C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
9. This kit should be stored at 2-8 °C.

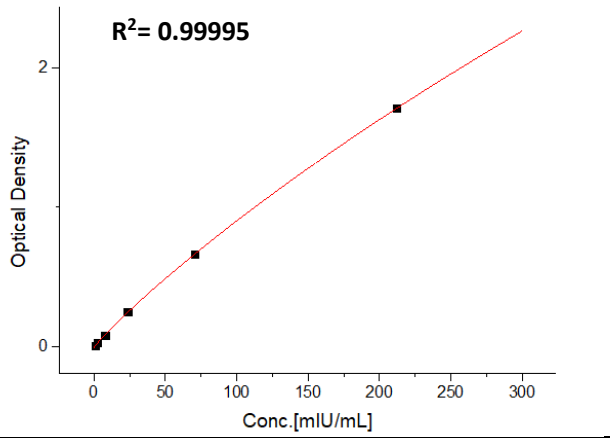
QUICK GUIDE


Quick Guide
1
Prepare
Prepare all reagents, standard curve, and samples as instructed.
2
Sample & detection antibody
Add test sample mix to wells. (Calibrator、samples、Biotin-Ab Solution)
18-25°C 2.0 hour
Remove liquid and wash plate
3
Streptavidin-HRP Solution
Add enzyme conjugated Streptavidin
18-25°C 30 min avoid light
Remove liquid and wash plate
4
Substrate Reaction
Colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.
18-25°C 15 min avoid light
5
Termination +Analysis
Add Stop solution and read absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

TYPICAL DATA

Note: *The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

EPO Standard (mIU/mL)	$OD_{450nm-630nm}$
212.00	1.727
70.67	0.681
23.56	0.269
7.85	0.096
2.62	0.045
0.87	0.026
Blank	0.018



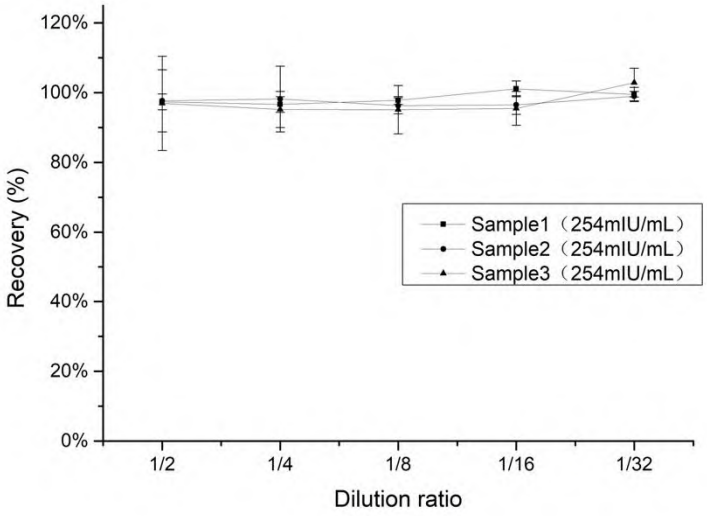
PERFORMANCE CHARACTERISTICS

1. Sensitivity

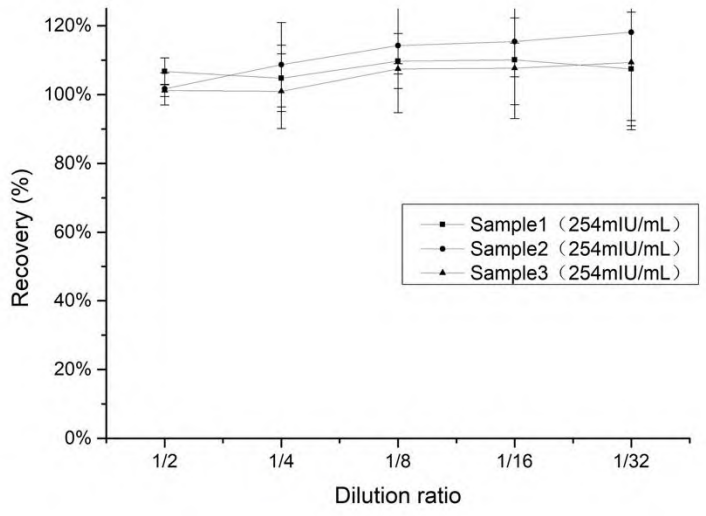
The minimum detectable concentration of EPO is less than 0.67 mIU/mL.

2. Linearity

Three samples (Serum) spiked with high concentrations of EPO were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of EPO for serum samples is 97.72%.



Three samples (EDTA plasma) spiked with high concentrations of EPO were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of EPO for serum samples is 108.25%.



3. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 μg/mL.

Human	IL-1β，IL-2，IL-4，IL-5，IL-6，IL-7，IL-8，IL-12 p70，IL-10、IL-10、MCP-1、M-CSF、TNF-α、IFN-γ

4. Intra-Assay Precision

Ten replicates of each of 4 samples containing different EPO concentrations were tested in one assay. Acceptable criteria: CV＜10%.

Sample Concentration (mIU/mL)	Mean (mIU/mL)	SD	Numbers	CV
101	114.3526	4.571078	10	4%
25.4	28.30166	0.7577	10	3%
12.7	13.49605	0.378961	10	3%
6.35	6.506036	0.344566	10	5%

5. Inter-Assay Precision

Five samples containing different concentrations of EPO were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (mIU/mL)	Mean (mIU/mL)	SD	Numbers	CV
212	212.1596917	0.149100335	9	0.07%
70.67	70.59590667	0.056852205	9	0.08%
23.56	23.62422667	0.130880068	9	0.55%
7.85	7.716932778	0.123343872	9	1.60%
2.62	2.642397778	0.095445041	9	3.59%

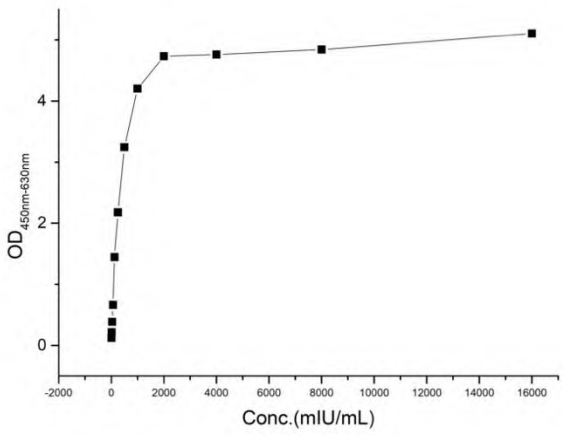
6. Recovery

Recombinant EPO was spiked into 3 human serum samples, and then analyzed. The average recovery of EPO for serum samples is 93.72%.

Sample ID	Conc Measured (mIU/mL)	Conc Added (mIU/mL)	Conc Recovered (mIU/mL)	Recovery
1	26.61	21.00	19.12	91.07%
	13.50	6.35	6.01	94.71%
	8.32	-		
2	27.71	21.00	18.39	87.56%
	15.50	6.35	6.18	97.36%
	10.35	-		
3	24.55	21.00	18.65	88.81%
	12.43	6.35	6.53	102.82%
	6.55	-		

7. Hook Effect

Not be affected by the concentration of EPO up to 2000 mIU/ml.



8. Interference effect

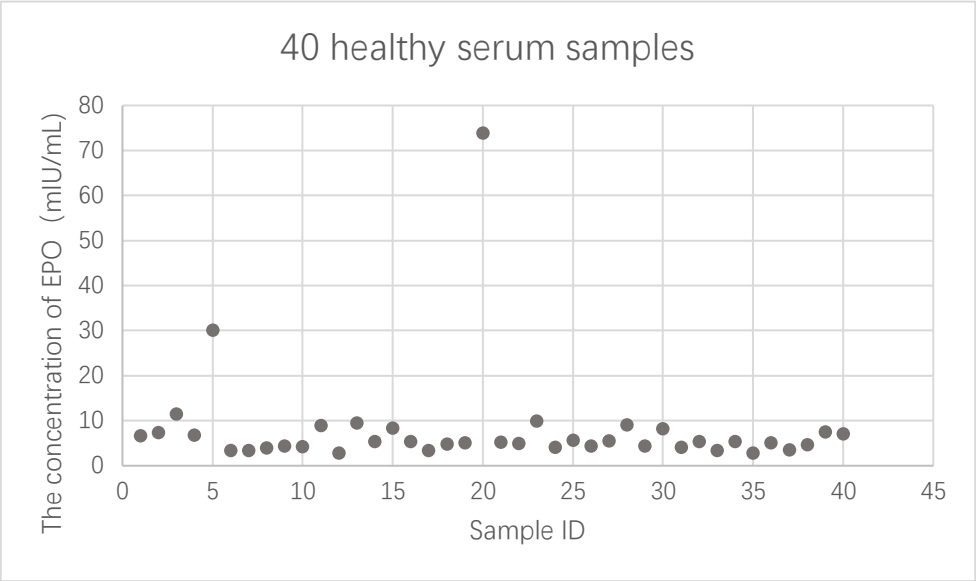
To evaluate the hemolysis matrix effect and high-dose triglyceride matrix effect of assay, serum samples spiked with high concentrations of hemoglobin (2%), or triglyceride (3 mg/mL) were tested. Results shown that all spiked analytes had recoveries between 89% and 117%, no hemolysis matrix effect and high-dose triglyceride matrix effect was observed in assay.

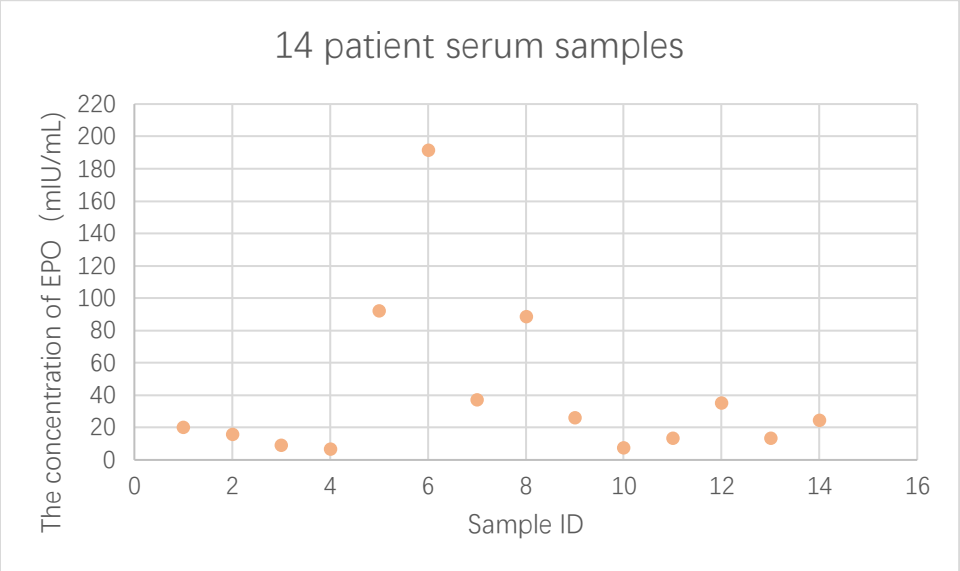
Spiked Material	ID	Conc-1(mIU/mL)	Conc-2(mIU/mL)	Mean(mIU/mL)	Recovery
2% Hemoglobin (v/v)	Sample 1	11.25	10.69	10.97	98%
	Spiked Sample1	10.56	10.89	10.73	
	Sample 2	10.64	9.80	10.22	90%
	Spiked Sample 2	9.44	9.00	9.22	
	Sample 3	2.62	2.91	2.76	110%
	Spiked Sample 3	3.11	2.95	3.03	
	Sample 4	3.76	3.56	3.66	106%
	Spiked Sample 4	4.13	3.64	3.89	

Spiked material	ID	Conc-1 (mIU/mL)	Conc-2 (mIU/mL)	Mean (mIU/mL)	Recovery
Triglyceride (3 mg/mL)	Sample 1	11.25	10.69	10.97	117%
	Spiked Sample1	12.66	12.95	12.81	
	Sample 2	10.64	9.80	10.22	92%
	Spiked Sample 2	9.80	8.96	9.38	
	Sample 3	2.62	2.91	2.76	104%
	Spiked Sample 3	2.95	2.79	2.87	
	Sample 4	3.76	3.56	3.66	89%
	Spiked Sample 4	3.28	3.28	3.28	

9. Sample values

40 healthy serum samples and 14 patient serum samples were evaluated for the concentrations of human EPO in assay.





TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts