Human EPO Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00153

Size: 96T

Sensitivity: 0.2 mIU/mL

Range: 4.7-300 mIU/mL

Usage: For the quantitative detection of human Erythropoietin concentrations in serum and plasma.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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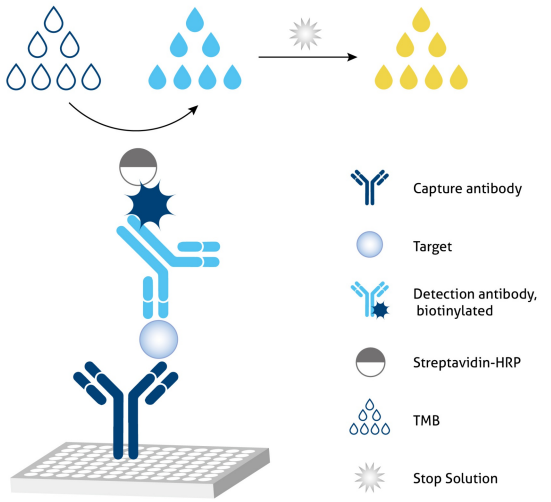
1. Background

Erythropoietin (EPO) is a glycoprotein hormone that regulates the production of red blood cells and biosynthesis of hemoglobin. The predominant expression of this gene shifts from the liver during fetal development to kidney in adults, and and the secreted protein will travel through the blood stream to reach to the bone marrow to stimulate hematopoietic stem cell differentiation to RBC. EPO binds to the cognate EPO receptor (EPOR) on erythroid progenitor cells, thus preventing apoptosis and stimulating their differentiation and maturation into erythrocyte. However, EPO protein and its receptors have also been shown to be cytoprotective in extra-hematopoietic tissues including the retina tissue. Low levels of EPO (around 10 mIU/mL) are constantly secreted sufficient to compensate for normal red blood cell turnover. Common causes of cellular hypoxia resulting in elevated levels of EPO (up to 10000 mIU/mL) include any anemia, and hypoxemia due to chronic lung disease.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 300 mIU/bottle; lyophilized	2 bottles	
Detection Antibody, biotinylated (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 4-af - 30 mL/bottle. For serum	1 bottle	
Sample Diluent PT 3-af - 30 mL/bottle. For plasma	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for serum and plasma; 1:2 is recommended for cell culture supernatant .

7.5 Standard Serial Dilution: Add 1 mL Sample Diluent PT 4-af or PT 3-af in protein standard. This reconstitution gives a stock solution of 300 mIU/mL.

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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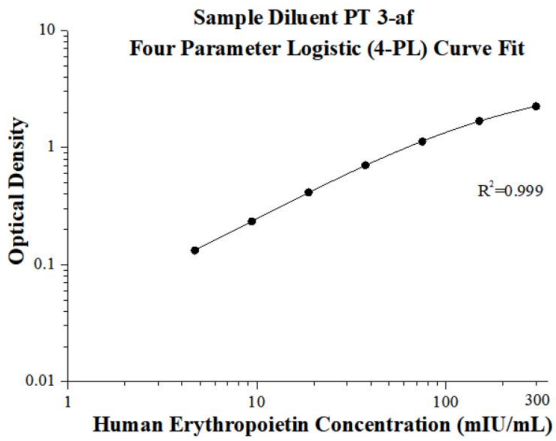
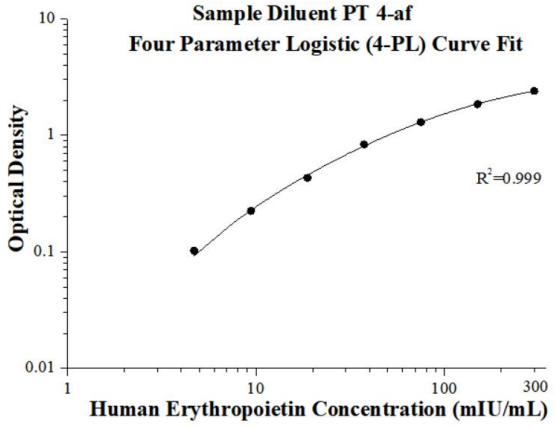
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(mIU/mL)	O.D	Average	Corrected
0	0.039 0.039	0.039	-
4.7	0.171 0.171	0.171	0.132
9.4	0.272 0.273	0.273	0.234
18.8	0.448 0.457	0.453	0.414
37.5	0.750 0.739	0.745	0.706
75	1.143 1.181	1.162	1.123
150	1.694 1.747	1.721	1.682
300	2.271 2.298	2.285	2.246



(mIU/mL)	O.D	Average	Corrected
0	0.047 0.045	0.046	-
4.7	0.151 0.145	0.148	0.102
9.4	0.273 0.267	0.270	0.224
18.8	0.471 0.483	0.477	0.431
37.5	0.878 0.880	0.879	0.833
75	1.351 1.330	1.341	1.295
150	1.938 1.845	1.892	1.846
300	2.421 2.466	2.444	2.398

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (mIU/mL)	SD	CV%
1	20	7.0	0.54	7.7
2	20	32.2	1.10	3.4
3	20	137.4	3.99	2.9

Inter-assay Precision				
Sample	n	Mean (mIU/mL)	SD	CV%
1	24	7.5	0.61	8.2
2	24	31.9	2.94	9.2
3	24	129.2	13.59	10.5

9.3 Recovery

The recovery of Erythropoietin spiked to three different levels in four samples throughout the range of the assay in human samples were evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	83	71-97
	1:4	94	81-107
Human plasma	1:2	94	72-106
	1:4	89	82-100

9.4 Sample values

Samples from healthy volunteers were evaluated for Erythropoietin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Range (mIU/mL)	Mean of Sample (mIU/mL)
Human serum (n=16)	8.1-43.3	23.4
Human plasma (n=16)	0.3-14.9	3.7

9.5 Sensitivity

The minimum detectable dose of human Erythropoietin is 0.2 mIU/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of Erythropoietin in in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma (Sample Diluent PT 3-af)	Human serum (Sample Diluent PT 4-af)
1:2	Average% of Expected	103	93
	Range (%)	101-106	84-106
1:4	Average% of Expected	103	98
	Range (%)	102-103	89-107
1:8	Average% of Expected	105	103
	Range (%)	96-114	95-116
1:16	Average% of Expected	100	108
	Range (%)	86-119	95-119

10. References

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2. Caprara C. et al.(2014) Mol Vis. 20:307-24.
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