

# Human EPO Sandwich ELISA Kit Datasheet

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

#### **General Information**

Catalogue Number	KE00153
Product Name	Human EPO Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	4.7-300 mlU/mL
Tested applications	Quantification ELISA

#### **Database Links**

Entrez Gene	2056
SwissProt	P01588

#### Kit Components & Storage

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

#### NB: Do not use the kit after the expiration date.

Sample Diluent PT 4-af is for protein standard and serum.

Sample Diluent PT 3-af is for protein standard and plasma.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1 mL Sample Diluent PT 4-af or PT 3-af in protein standard. This reconstitution gives a stock solution of 300 mlU/mL.



#### **Product Description**

KE00153 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Erythropoietin ELISA kit is to be used to detect and quantify protein levels of endogenous Erythropoietin. The assay recognizes mouse Erythropoietin. An antibody specific for Erythropoietin has been pre-coated onto the microwells. The Erythropoietin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse Erythropoietin is added to detect the captured mouse Erythropoietin protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

#### 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 mlU/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 mlU/mL) include any anemia, and hypoxemia due to chronic lung disease.

## Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. 1:2 or 1:4 dilution is recommended for serum or plasma.

## Safety Notes

This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

### Assay Procedure Summary

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	6 Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.					



## Example data

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



(mIU/mL)	0.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)	0.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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## 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						Inter-assay Precision			
Sample	n	Mean (mlU/mL)	SD	CV%	Sample	n	Mean (mlU/mL)	SD	CV%
1	20	7.0	0.54	7.7	1	24	7.5	0.61	8.2
2	20	32.2	1.10	3.4	2	24	31.9	2.94	9.2
3	20	137.4	3.99	2.9	3	24	129.2	13.59	10.5

#### 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 (%)
	1:2	83	71-97
	1:4	94	81-107
Human plasma	1:2	94	72-106
חטווומוו אנסצווומ	1:4	89	82-100

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

## Sensitivity

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

## 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
1.2	Range (%)	101-106	84-106
	Average% of Expected	103	98
1.4	Range (%)	102-103	89-107
1.0	Average% of Expected	105	103
1:8	Range (%)	96-114	95-116
1:16	Average% of Expected	100	108
	Range (%)	86-119	95-119

#### References

- 1. Chateauvieux S. et al. (2011) Biochem Pharmacol. 15;82(10):1291-303.
- 2. Caprara C. et al.(2014) Mol Vis. 20:307-24.
- 3. Brines M. et al. (2004) Proc Natl Acad Sci U S A. 101(41):14907-12.
- 4. Mancini DM. et al. (2003) Circulation. 107(2):294-9.
- 5. Shirley Ding SL. et al. (2016) Eye (Lond). 30(10):1293-1309.

