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# **EPOR ELISA Kit**





#### Overview

Quantity:	96 tests
Target:	EPOR
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	180-12.000 pg/mL
Minimum Detection Limit:	180 pg/mL
Application:	ELISA

Product Details	
Purpose:	Human Erythropoietin R ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin,
	BDNF, BLC, CNTF, ENA-78, FGF-4, IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12
	p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, FGF-4, FGF-6, FGF-7, G-CSF, GDNF, GM-CSF, IFN-g,
	IGFBP-2, IGFBP-3, IGFBP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG, MIP-1a, MIP-1
	b, MIP-1 delta, PARC, PDGF, RANTES, SCF, SDF-1 alpha, TARC, TGF-b, TIMP-1, TIMP-2, TNF-a,
	TNF-b, TPO, VEGF.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin,
	BDNF, BLC, CNTF, ENA- 78, FGF-4, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-

	11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, FGF-4, FGF-6, FGF-7, G-CSF, GDNF, GM-CSF,
	IFN-gamma, IGFBP-2, IGF-BP-3, IGF-BP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG,
	MIP-1alpha, MIP-1 beta, MIP- 1delta, PARC, PDGF, RANTES, SCF,SDF-1alpha, TARC, TGF-beta,
	TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Characteristics:	Strip plates and additional reagents allow for use in multiple experiments
	Quantitative protein detection
	Establishes normal range
	The best products for confirmation of antibody array data
Components:	Pre-Coated 96-well Strip Microplate
	Wash Buffer
	Stop Solution
	Assay Diluent(s)
	Lyophilized Standard
	Biotinylated Detection Antibody
	Streptavidin-Conjugated HRP
	TMB One-Step Substrate
Material not included:	Distilled or deionized water
	<ul> <li>Precision pipettes to deliver 2 μL to 1 μL volumes</li> </ul>
	<ul> <li>Adjustable 1-25 µL pipettes for reagent preparation</li> </ul>
	• 100 µL and 1 liter graduated cylinders
	Tubes to prepare standard and sample dilutions
	Absorbent paper

### **Target Details**

Target:	EPOR
Alternative Name:	EPO R (EPOR Products)
Gene ID:	2057
UniProt:	P19235
Pathways:	JAK-STAT Signaling

• Microplate reader capable of measuring absorbance at 450nm

· Log-log graph paper or computer and software for ELISA data analysis

### **Application Details**

Application Notes: Recommended Dilution for serum and plasma samples2 fold

### **Application Details**

Sample Volume:	100 μL
Plate:	Pre-coated
Protocol:	<ol> <li>Prepare all reagents, samples and standards as instructed in the manual.</li> <li>Add 100 μL of standard or sample to each well.</li> <li>Incubate 2.5 h at RT or O/N at 4 °C.</li> <li>Add 100 μL of prepared biotin antibody to each well.</li> <li>Incubate 1 h at RT.</li> <li>Add 100 μL of prepared Streptavidin solution to each well.</li> <li>Incubate 45 min at RT.</li> <li>Add 100 μL of TMB One-Step Substrate Reagent to each well.</li> <li>Incubate 30 min at RT.</li> <li>Add 50 μL of Stop Solution to each well.</li> <li>Read at 450 nm immediately.</li> </ol>

#### Reagent Preparation:

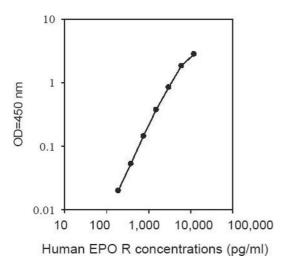
1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, 1x Assay Diluent D (Item K) should be used for dilution of serum/plasma /culture supernatants/urine. The Human EPO R ELISA Kit Protocol 3 Suggested dilution for normal serum/plasma: 2 fold\*. \* Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL 1x Assay Diluent D (Item K, Assay Diluent D should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 120 µL of the EPO R standard from the vial of Item C, into a tube with 380 µL 1x Assay Diluent D to prepare a 12,000 pg/ml standard solution. Pipette 250 µL 1x Assay Diluent D into each tube. Use 12,000 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent D serves as the zero standard (0 pg/ml). 200 µL 200 µL 200 μL 200 μL 200 μL 120 μL standard + 380 μL 200myl 12,000 6,000 3,000 1,500 750 375 187.5 0 pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 The Human EPO R ELISA Kit Protocol 4 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-

	Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-
	Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B (Item E). For
	example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1x Assay Diluent B to prepare a 300-fold
	diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is
Assay Procedure.	recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of
	each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well
	and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking. 3.
	Discard Pipette or autowasher. Complete removal of liquid at each step is essential to good
	performance. After the last wash, remove any remaining Wash Buffer by aspirating or
	decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of 1x prepared
	biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room
	temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add
	100 μL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well.
	Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution.
	Repeat the wash as in step 3. 8. Add 100 $\mu L$ of TMB One-Step Substrate Reagent (Item H) to
	each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add
	$50~\mu L$ of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and
	subtract the average zero standard optical density. Plot the standard curve on log-log graph
	paper or using Sigma plot software, with standard concentration on the x-axis and absorbance
	on the y-axis. Draw the best-fit straight line through the standard points.
Assay Precision:	Intra-Assay: CV<10%
	Inter-Assay: CV<12%
Restrictions:	For Research Use only
Handling	
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated
	freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is
	recommended to store at -80°C.

Expiry Date:

6 months

### **Images**



## ELISA

Image 1.