Mouse EPO Immunoassay

Catalog Number: SEKM-0157

For the quantitative determination of Mouse EPO concentrations in cell culture supernate, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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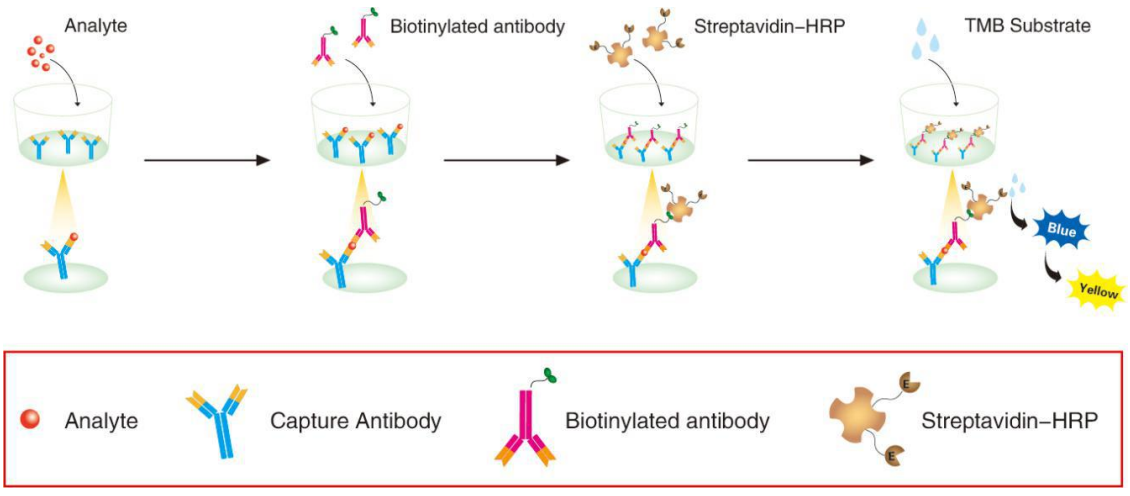
BACKGROUND

Erythropoietin (EPO) is a 34-39 kDa secreted glycoprotein that is a member of the type I cytokine superfamily. The mouse EPO gene encodes a 192 amino acid (aa) residue precursor that contains a 26 aa signal peptide and a 166 aa mature protein containing three potential N-linked glycosylation sites. Mouse EPO lacks the O-linked glycosylation site found in human EPO. Although carbohydrate chains are not required for in vitro receptor binding, they are required for in vivo EPO bioactivity. Depending on the cell source, different EPO isoforms are produced that differ in their glycan compositions and sialic acid contents. Mature mouse and rat EPO share 94% aa sequence identity. They also share from 80%-82% aa identity with mature human, porcine, rhesus monkey and feline EPO . EPO is primarily produced by cells in the kidney (interstitial peritubular renal fibroblasts) and liver (hepatocytes and Ito cells), where its production is up-regulated by hypoxia. Other tissues and cells, including neural tissues (astrocytes and neurons), testis (Sertoli cells), uterus, placenta, and erythroid progenitors, have also been shown to produce EPO.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific forEPO has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and anyEPO present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific forEPO is added to detect the capturedEPO protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. 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 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized, 16000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernate - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

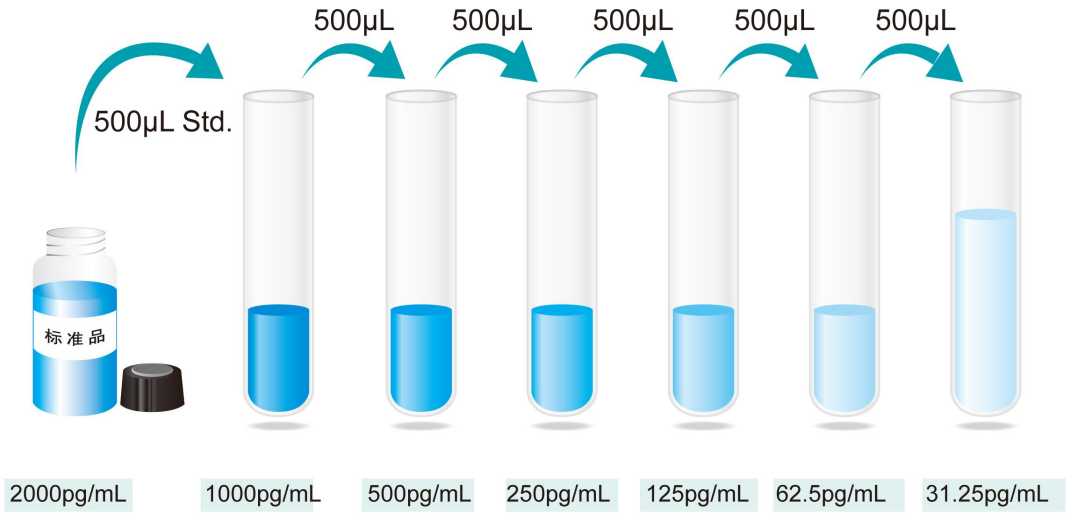
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer.

The 2000 pg/mL standard serves as the high standard. The Standard/specimen Diluent serves as the zero standard (0 pg/mL).



Preparation of EPO standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-MouseEPO antibody(1 vial)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 55 μL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 100μL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-mouse EPO antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 15 minutes,37℃.Protect from light.

⇩

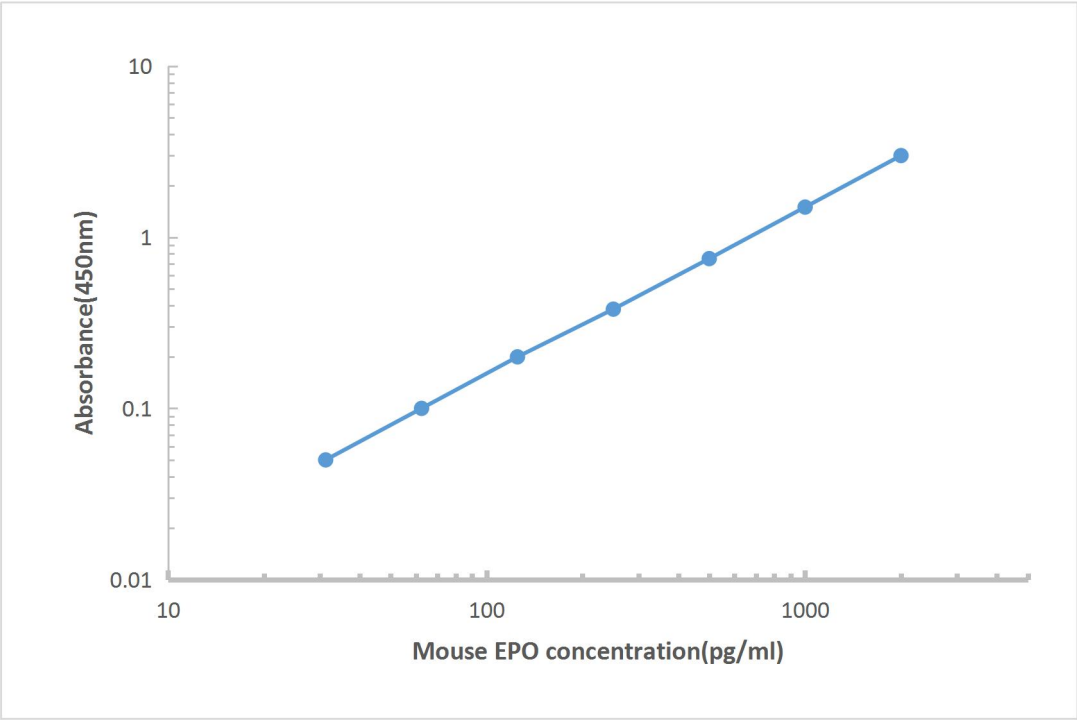
Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the EPO concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using theEPO ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.041	0.045	0.043	---
31.25	0.243	0.244	0.244	0.201
62.5	0.281	0.282	0.281	0.238
125	0.418	0.419	0.418	0.375
250	0.663	0.665	0.664	0.621
500	1.077	1.081	1.079	1.036
1000	1.754	1.759	1.757	1.713
2000	2.858	2.866	2.862	2.819



Representative standard curve for EPO ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouseEPO. The following recombinant Mouse proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
ApoAI, BMP1, BMP2, BMP3, BMP4, BMP7, CRP, HGF, HSP27, IL-1α, IL-1 RI, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-21, IL-23, IFN , MMP-2, sIL-2R, sIL-6R, PDGF, PLA2G7, prolactin, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of EPO spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of EPO in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	97	90－104
Cell culture supernatants	102	94－110

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of EPO in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	97	102
	Range (%)	90－104	94－109
1:4	Average% of Expected	96	107
	Range (%)	87－103	99－114
1:8	Average% of Expected	98	100
	Range (%)	92－106	93－107
1:16	Average% of Expected	101	104
	Range (%)	94－108	95－112

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