



LEGEND MAX™
ELISA Kit



Rat Erythropoietin

Cat. No. 442807

ELISA Kit for Accurate Quantitation of Rat
Erythropoietin from Cell Culture Supernatant,
Serum and Other Biological Fluids

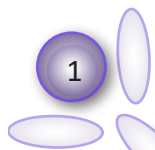
BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.



| Table of Contents | Page |
|---|-------------|
| Introduction..... | 2 |
| Materials Provided..... | 2 |
| Materials to be Provided by the End-User..... | 3 |
| Storage Information..... | 3 |
| Health Hazard Warnings..... | 3 |
| Specimen Collection and Handling..... | 4 |
| Reagent and Sample Preparation..... | 4 |
| Assay Procedure..... | 5 |
| Assay Procedure Summary..... | 7 |
| Calculation of Results..... | 8 |
| Typical Data..... | 8 |
| Performance Characteristics..... | 9 |
| Specificity..... | 9 |
| Sensitivity..... | 9 |
| Recovery..... | 9 |
| Linearity..... | 9 |
| Intra-Assay Precision..... | 9 |
| Inter-Assay Precision..... | 9 |
| Biological Samples..... | 10 |
| Troubleshooting Guide..... | 11 |
| ELISA Plate Template..... | 13 |



LEGEND MAX™ Rat EPO ELISA Kit

Introduction:

Erythropoietin (EPO) is the most important hormone regulating erythropoiesis. It is a glycoprotein also known as hematopoietin. EPO is mainly produced by kidney and liver in response to hypoxia to induce red blood cell production. Rat EPO is a secreted protein of 166 amino acids with a predicted molecular mass of ~18.5 kD. However, it migrates on SDS-PAGE at ~35-50 kD due to heavy glycosylation. Rat EPO is closely related to thrombopoietin (TPO) and highly conserved across species, with rat EPO being 95% and 79% identical to mouse and human, respectively.

EPO functions through binding to its receptor, EPO R, a homodimer, on erythroid precursors, and promotes their differentiation and maturation. As expected, serum levels of EPO are elevated in response to various conditions that result in tissue hypoxia (e.g., blood loss, high altitude, cardiac or pulmonary disease, and anemia). Increased EPO levels can also be due to increased production by cancer cells of the liver and kidney. Decreased levels of EPO are often seen with chronic renal failure and other chronic diseases or chemotherapy, which causes anemia. Rat endogenous EPO and recombinant EPO share the same amino acid sequence, with differences in the glycosylation profiles.

The LEGEND MAX™ Rat EPO ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal rat anti-rat EPO antibody. The Detection Antibody is a biotinylated polyclonal goat anti-rat EPO antibody. This kit is specifically designed for the accurate quantitation of rat EPO from cell culture supernatant, serum, tissue homogenates and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

| Description | Quantity | Volume (per bottle) | Part # |
|--|----------|------------------------|--------|
| Anti-rat EPO Pre-coated 96 well Strip Microplate | 1 plate | | 77898 |
| Rat EPO Detection Antibody | 1 bottle | 12 mL | 77899 |
| Rat EPO Standard | 1 vial | lyophilized | 77901 |
| Avidin-HRP | 1 bottle | 12 mL | 77897 |
| Assay Buffer A | 1 bottle | 25 mL | 78232 |
| Wash Buffer (20X) | 1 bottle | 50 mL | 78233 |
| Substrate Solution F | 1 bottle | 12 mL | 79132 |
| Stop Solution | 1 bottle | 12 mL | 79133 |
| Plate Sealers | 4 sheets | | 78101 |

LEGEND MAX™ Rat EPO ELISA Kit

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

| Opened or Reconstituted Components | |
|------------------------------------|---|
| Microplate wells | If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month. |
| Standard | The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles. |
| Detection Antibody | Store opened reagents between 2°C and 8°C and use within one month. |
| Avidin-HRP | |
| Assay Buffer A | |
| Wash Buffer (20X) | |
| Substrate Solution F | |
| Stop Solution | |

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with

LEGEND MAX™ Rat EPO ELISA Kit

NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer. *Assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.*

Plasma: Collect blood samples in heparin-containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. *Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.*

Tissue Homogenates: Using an appropriately sized homogenizer, homogenate one rat liver and two kidneys into 6 mL and 2 mL, respectively, of cold 1XPBS containing protease inhibitors. After two rounds of freeze-thaw cycles, centrifuge tissue homogenates, collect supernatant, and store at < -70°C. *Once sample is ready for assay, avoid repeated freeze-thaw cycles.*

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

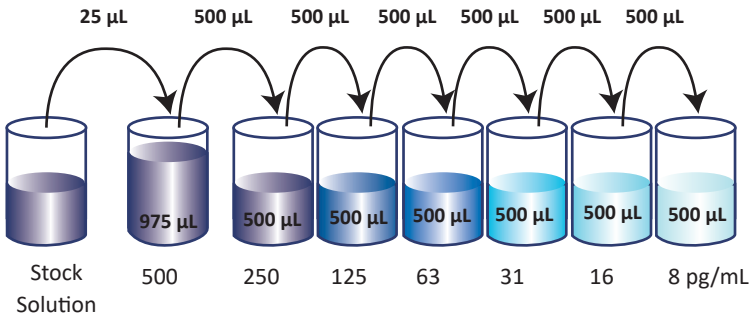
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and mix until dissolved.
2. Reconstitute the lyophilized Rat EPO Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilution is necessary, samples should be diluted in the corresponding cell culture medium.
4. It is recommended that normal rat serum samples be analyzed with no dilution. However, if dilution is required, samples should be diluted with Assay Buffer A.

LEGEND MAX™ Rat EPO ELISA Kit

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 1,000 μL of the 500 pg/mL top standard by diluting 25 μL of the standard stock solution in 975 μL of Assay Buffer A. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes, using Assay Buffer A as the diluent. Thus, the rat EPO standard concentrations in the tubes are 500 pg/mL , 250 pg/mL , 125 pg/mL , 63 pg/mL , 31 pg/mL , 16 pg/mL and 8 pg/mL , respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. For measuring samples:
 - a) Add 50 μL of Assay Buffer A to each well that will contain either standards or samples.
 - b) Add 50 μL of standard dilutions to the wells for standards. Add 50 μL of samples to the wells for samples.
5. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
6. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer. Wash the plate with at least 300 μL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
7. Add 100 μL of Rat EPO Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

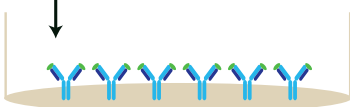
LEGEND MAX™ Rat EPO ELISA Kit


8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 6.
9. Add 100 μL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
10. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 6. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
11. Add 100 μL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing rat EPO should turn blue in color with intensity proportional to concentration. It is not necessary to seal the plate during this step.
12. Stop the reaction by adding 100 μL of Stop Solution to each well. The well color should change from blue to yellow.
13. Read absorbance at 450 nm within 20 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

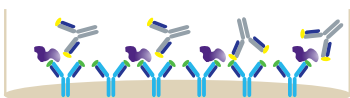
LEGEND MAX™ Rat EPO ELISA Kit

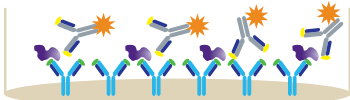
Assay Procedure Summary

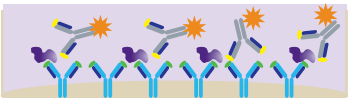
1. Add 50 μL Assay Buffer A

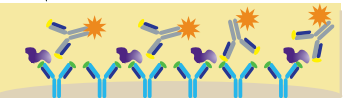

2. Add 50 μL prepared standards or samples
Incubate 2 hrs, RT, shaking


3. Wash 4 times
Add 100 μL Detection Antibody solution
Incubate 1 hr, RT, shaking


4. Wash 4 times
Add 100 μL Avidin-HRP solution
Incubate 30 min, RT, shaking


5. Wash 5 times
Add 100 μL Substrate Solution F
Incubate 10 min, RT, in the dark


6. Add 100 μL Stop Solution


7. Read absorbance at 450 nm and 570 nm

LEGEND MAX™ Rat EPO ELISA Kit

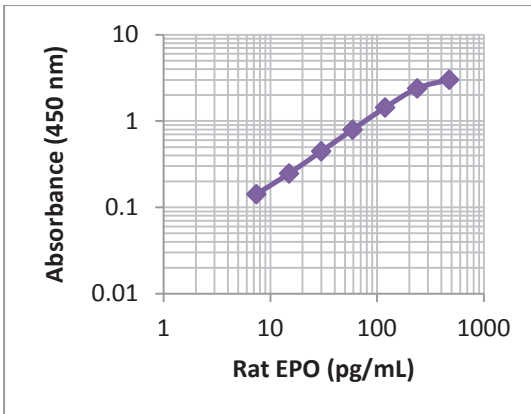
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with antigen concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown antigen concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the sample concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



LEGEND MAX™ Rat EPO ELISA Kit

Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at 50 ng/mL. This kit has high cross-reactivity with human EPO and mouse EPO.

| | |
|-------|---|
| Rat | TPO, IFN- γ , IL-33, IL-22, IL-4, CCL20, TNF- α , CX3CL1, GM-CSF, IGF-1, IL-2, IL-17A, IL-6 |
| Mouse | EPO R, TPO |
| Human | EPO R, TPO |

Sensitivity: The average minimum detectable concentration of rat EPO is 1.0 pg/mL.

Recovery: Three levels of recombinant rat EPO (375 pg/mL, 94 pg/mL and 23 pg/mL) were spiked into four rat serum samples (each serum was pooled from minimum ten rats of the same stain, four strains) and analyzed with the LEGEND MAX™ Rat EPO ELISA Kit. On average, 88% of the spiked protein was recovered from serum samples.

Linearity: Four rat serum samples (two were diluted directly, two were spiked with recombinant rat EPO at 500 pg/mL prior to dilutions) were diluted with Assay Buffer A to produce sample concentrations within the dynamic range of the assay. On average, 108% of the expected level was detected from serum samples.

Intra-Assay Precision: Two samples with different concentrations of rat EPO were tested with sixteen replicates in one assay.

| | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Replicates | 16 | 16 |
| Mean Concentration (pg/mL) | 255.0 | 13.4 |
| Standard Deviation | 17.1 | 0.8 |
| % CV | 6.7 | 5.9 |

Inter-Assay Precision: Two samples with different concentrations of rat EPO were assayed in four independent assays.

| | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Assays | 4 | 4 |
| Mean Concentration (pg/mL) | 256.3 | 13.6 |
| Standard Deviation | 19.7 | 0.8 |
| % CV | 7.7 | 5.7 |

LEGEND MAX™ Rat EPO ELISA Kit

Biological Samples:

Serum

Normal rat serum samples pooled from minimum ten rats of each strain (four strains) were tested for endogenous EPO. The concentrations (pg/mL) measured are shown below:

| Strain | Serum |
|-----------------|-------|
| Wistar Hannover | 31.0 |
| Sprague Dawley | 25.0 |
| Fischer 344 | 19.5 |
| Long Evans | 19.0 |

Tissue Homogenates from rat anemia model

Sprague Dawley rat (female, seven months old) was bled, 3 mL blood via retro-orbital plexus. The rat was sacrificed 24 hours later and blood, liver and kidney were isolated. Plasma and tissue homogenates were prepared following instructions in “Specimen Collection and Handling” section. Total protein concentrations were determined by Bradford Protein Assay (mg/ml). Samples were tested with the LEGEND MAX™ Rat EPO ELISA Kit. The concentrations of rat EPO in pre-anemic and post-anemic plasma and tissue homogenates are shown below.

| | Pre-anemia | Post-anemia |
|----------------------------|------------|-------------|
| Plasma (pg/mL) | 8.9 | 1675.5 |
| Kidney Homogenates (pg/mg) | 1.4 | 19.9 |
| Liver Homogenates (pg/mg) | 0.2 | 0.6 |

Troubleshooting Guide:

| Problem | Probable Cause | Solution |
|-----------------------------------|--|---|
| High Background | Background wells were contaminated | Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents. |
| | Insufficient washes | Increase number of washes. Increase soaking time between washes prior to addition of substrate solution. |
| | TMB Substrate Solution was contaminated | TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells. |
| No or poor signal | Detection Antibody, Avidin-HRP or Substrate solution were NOT added | Rerun the assay and follow the protocol. |
| | Wrong reagent or reagents were added in wrong sequential order | |
| | Insufficient plate agitation | The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing. |
| | The wash buffer contains Sodium Azide (NaN ₃) | Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity. |
| | Incubations were done at an inappropriate temperature, timing or without agitation | Rerun the assay and follow the protocol. |
| Low or poor standard curve signal | The standard was incorrectly reconstituted or diluted | Adjust the calculations and follow the protocol. |
| | Standard was inappropriately stored | Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles. |
| | Reagents added to wells with incorrect concentrations | Check for pipetting errors and the correct reagent volume. |

LEGEND MAX™ Rat EPO ELISA Kit

| Problem | Probable Cause | Solution |
|---|---|---|
| Signal is high, standard curves have saturated signal | Standard reconstituted with less volume than required | Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol. |
| | Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long | Rerun the assay and follow the protocol. |
| Sample readings are out of range | Samples contain no or below detectable levels of the analyte | If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications. |
| | Samples contain analyte concentrations greater than highest standard point | Samples may require dilution and analysis. |
| High variation in samples and/or standards | Multichannel pipette errors | Confirm that pipette calibrations are accurate. |
| | Plate washing was not adequate or uniform | Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps. |
| | Non-homogenous samples | Thoroughly mix samples before assaying. |
| | Samples may have high particulate matter | Remove particulate matter by centrifugation. |
| | Cross-well contamination | Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate. |



| ELISA Plate Template | | | | | | | | | | | | |
|----------------------|---|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |



LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**

8999 BioLegend Way

San Diego, CA 92121

Tel: 1.858.768.5800

Tel US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)

Fax: 1.877.455.9587

Email: info@biolegend.com

biolegend.com

For a complete list of world-wide BioLegend offices and distributors,
please visit our website at: biolegend.com