Manufactured and Distributed by:

Novatein Biosciences

310 W Cummings Park, MA, 01810, USA

Phone: (617) 238-1396

Fax: (617) 380-0053

Toll Free: (888) 856-2858

http://www.novateinbio.com/

Email: Info@novateinbio.com

NOVATEINBIO

Accelerates Research and Development

Rat Erythropoietin, EPO ELISA Kit

Catalog number: NB-E30560 (96 wells)

The kit is designed to detect the level of Rat EPO in cell culture supernatant, serum, plasma and other suitable sample solution

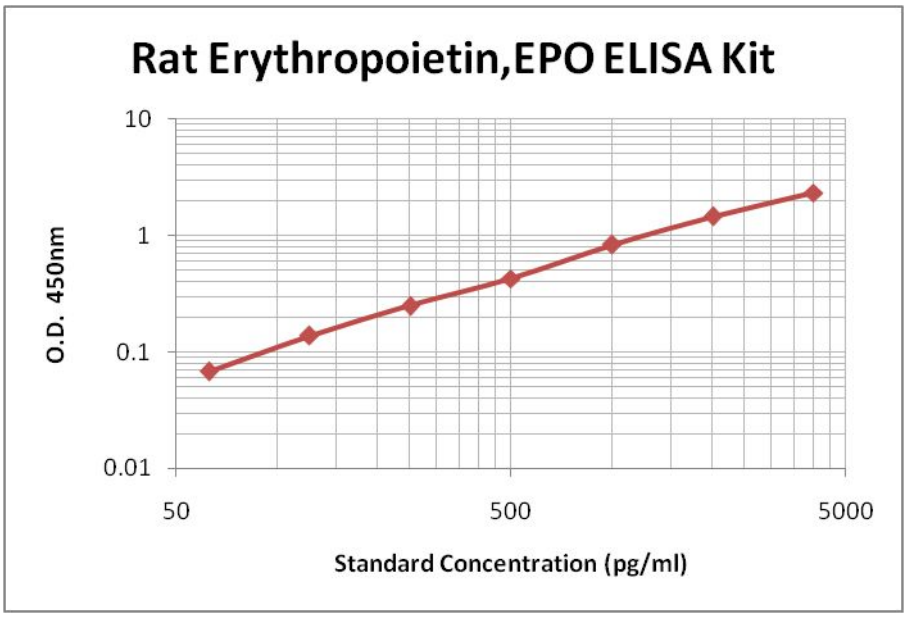
FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before the test, please read this manual carefully; After reading the subsequent contents of this manual, please note the following specially :

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over-develop)
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

Typical data:



Background:

Erythropoietin (Epo), also known as hematopoietin or hemopoietin, is a secreted glycoprotein belonging to member of the type I cytokine superfamily. The mouse Epo precursor protein consists of a 26 aa signal peptide and a 166 aa mature protein with three potential N-linked glycosylation sites. Epo is primarily produced in interstitial peritubular renal fibroblasts in the kidney and hepatocytes and Ito cells in liver in addition to other tissues and cells, such as astrocytes and neurons, testis (Sertoli cells), uterus, placenta, and erythroid progenitors.

Epo plays an essential role in red cell production. Under hypoxic conditions, the kidney will produce and secrete Epo to increase the production of red blood cells by targeting CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation. Primarily, Epo promotes survival of cell progenitors and precursors by protecting these cells from apoptosis. Apart from its role in erythropoiesis, Epo also functions as a viability and proliferation factor in other cells. For example, Epo can stimulate myoblast proliferation while suppressing its differentiation, resulting in the expansion of the progenitor cell population. Epo protects against ischemic and toxic injuries to neuronal, cardiovascular and renal tissues. Epo has also been shown to promote angiogenesis under physiologic and pathologic conditions. Epo binds to homodimeric Epo receptor (Epo R) to activate the downstream signaling cascade.

Erythropoietins have been produced by recombinant DNA technology to be used as therapeutic agents in treating anemia resulting from chronic kidney disease, inflammatory bowel disease and myelodysplasia from chemotherapy and radiation in cancer patients.

Assay procedures

Bring all reagents to room temperature before use. Rat EPO Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Rat EPO concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 90 minutes at 37℃. Note: We recommend that each Rat EPO standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate 60minutes at 37℃.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at 37℃.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 90µl of TMB developing agent to each well, subsequently. Cover and incubate for 20-40 minutes at room temperature. (Protect from light. Do not over-develop).
8. Add 90µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat EPO concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

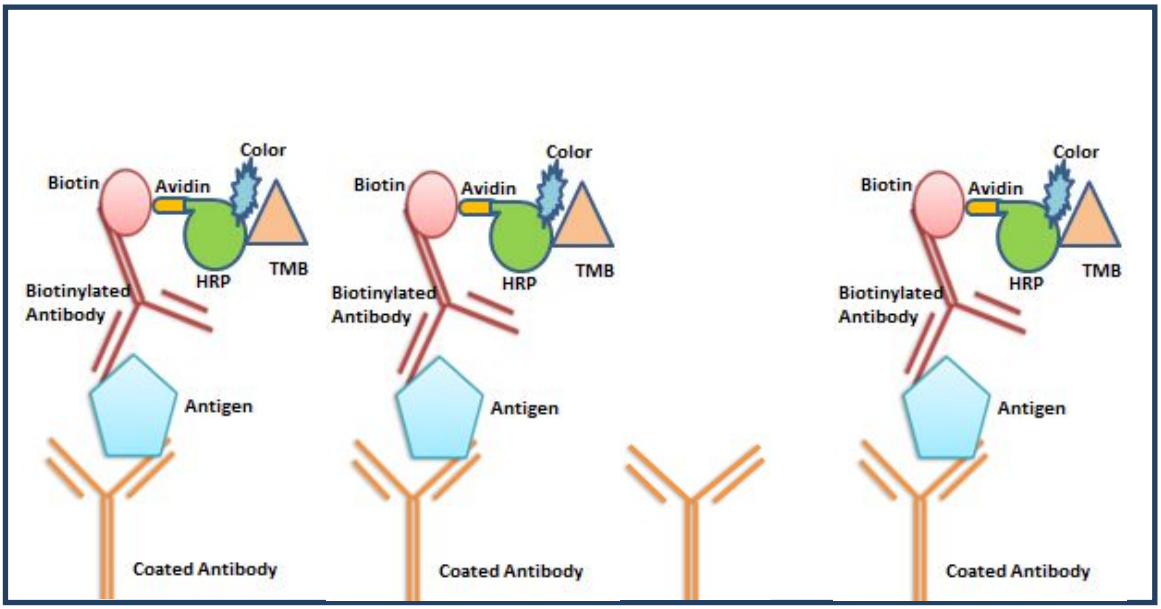
Intended use

The kit is used to quantify the Rat EPO in serum, plasma, body fluids, tissue lysate or cell culture supernatant.

Standard range	62.5 - 4000 pg/ml
Sensitivity	30 pg/ml
Assay time	4 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Rat EPO ELISA Kit was based on standard sandwich enzyme-linked immunosorbent assay technology. Rat EPO specific antibodies were precoated onto 96-well plates. The Rat EPO specific detection antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP was added and unbound conjugates were washed away with wash buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Rat EPO amount of sample captured in plate.



Materials supplied

1. Rat EPO standard:	10 ng/vial ×2.
2. One 96-well plate pre-coated with anti- Rat EPO Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Detection antibody:	130 µl, dilution 1:100.
5. Streptavidin-HRP:	130 µl, dilution 1:100.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. TMB developing agent:	10 ml.
9. Stop solution:	10 ml.
10. 20 × Wash Solution:	25 ml.
11. Plate sealer	1.
12. Package insert	1.

Materials required but not supplied

- 37℃ incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.

.Materials used for sample preparation.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Standard

Rat EPO: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (10ng /vial) are included in each kit. Use one tube for each experiment.

- 4000pg/ml→62.5 pg/ml of Rat EPO standard solutions:
- Add 1 ml of sample diluents into one standard tube with 10 ng Rat EPO. Keep the tube at room temperature for 10 minutes and mix thoroughly.This is 10000 pg/ml standard solution.
- Label 7 Eppendorf tubes with 4000pg/ml, 2000 pg/ml,1000 pg/ml,500pg/ml,250 pg/ml, 125pg/ml, 62.5 pg/ml respectively.Aliquot 0.6 ml of the sample diluents and add 0.4ml of 10000 pg/ml standard solution into 4000pg/ml tube.Then make 2-fold serial dilution from 4000 pg/ml to 62.5 pg/ml in seven 1.5 ml tubes. Make sure each tube has >= 300 ul standard.

Note: The standard solutions are best used within 2 hours.

Preparation of biotinylated anti- Rat EPO antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-Rat EPO antibody should be diluted in 1:100 with Antibody diluent buffer and mixed thoroughly.

Preparation of Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Streptavidin-HRP should be diluted in 1:100 with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.