Human Erythropoietin (EPO) ELISA Kit

Catalog No. CSB-E04538h

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of **human EPO** concentrations in **serum, plasma**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

Erythropoietin (EPO) is a hormone produced by the kidney that promotes the formation of red blood cells in the bone marrow. EPO is a glycoprotein (a protein with a sugar attached to it). The kidney cells that make EPO are specialized and are sensitive to low oxygen levels in the blood. These cells release EPO when the oxygen level is low in the kidney. EPO then stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood.

EPO is the prime regulator of red blood cell production. Its major functions are to promote the differentiation and development of red blood cells and to initiate the production of hemoglobin, the molecule within red cells that transports oxygen. EPO is produced not only in the kidney but also, to a lesser extent, in the liver. Different DNA sequences flanking the EPO gene act to control kidney versus liver production of EPO.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to EPO. Standards or samples are then added to the appropriate microtiter plate wells with a Horseradish Peroxidase (HRP)-conjugated antibody preparation specific for

EPO and incubated. Then substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of EPO in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

3.125 mIU/ml-100 mIU/ml. The standard curve concentrations used for the ELISA's were 100 mIU/ml, 50 mIU/ml, 21.875 mIU/ml, 9.375 mIU/ml, 3.125 mIU/ml.

SPECIFICITY

This assay recognizes human EPO. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of human EPO is typically less than 1.56 mIU/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard(S0-S5)	6 x 0.5 ml
HRP-conjugate	1 x 6 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml

Standard	S0	S1	S2	S3	S4	S5
Concentration (mIU/ml)	0	3.125	9.375	21.875	50	100

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.
2. Opened test plate should be stored at 2-8°C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Set a Blank well without any solution. Add 50µl of **Standard** or **Sample** per well.
2. Add 50µl of **HRP-Conjugate** to each well (Not to Blank!). Incubate for 1 hour at 37°C.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with **ddH_2O** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
5. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create 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 X-axis against the concentration on the Y-axis and draw a best fit curve through the points on the graph. 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.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless or light blue to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.