KRIBIOLISA™ EPO (Erythropoietin) ELISA

REF : KBBA01

Ver3.0

RUO

Enzyme Immunoassay for the quantitative determination of EPO (Erythropoietin) in serum, plasma and cell culture supernatant

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBBA01 Σ **96 tests**

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Introduction:

Erythropoietin (EPO) is a heavily glycosylated protein with a molecular weight of about 30,000 - 34,000 Daltons. EPO is a polypeptide consisting of 166 amino acids, containing one O-linked and three N-linked carbohydrate chains. The recombinant EPO is a good substitute for the native protein for use in an immunoassay.

Intended Use:

This kit is used for the quantitative determination of EPO (Erythropoietin) in serum, plasma and cell culture supernatant

Principle:

The method employs sandwich ELISA technique. Streptavidin is pre-coated onto microwells. Samples and standards are pipetted into microwells and EPO present in the sample are bound by the biotin labeled antibodies to the microwell. Anti-EPO-HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of EPO in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Streptavidin Microtiter Coated Plate (12 x 8 wells) - 1 no
2. Biotin Conjugated Antibody - 2.7 ml
3. HRP Conjugate - 2.7 ml
4. Standard (Lyophilized) - 0, 10, 20, 40, 110 mU/ml
5. Control 1 (Lyophilized) - 1 vial
6. Control 2 (Lyophilized) - 1 vial
7. Wash Buffer (20X) - 30 ml
8. TMB Substrate - 15 ml
9. Stop Solution - 20 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC and **Store the standards and controls at -20ºC or below after reconstitution. Standards and controls are stable at -20ºC for 6 weeks after reconstitution with up to 3 freeze thaw cycles**
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

2. For Research Use Only.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. Control 1 and Control 2: Reconstitute the controls in 2 ml of DI water. Allow the vials to sit for approximately 10 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use. Use the controls immediately after reconstitution.
4. Standards: Standard 0 mU /ml: Reconstitute the vial in 4.0 mL of DI water.
5. Other Standards: Reconstitute the vials in 2.0 mL of DI water.
6. Wash Buffer (1X): Add 30 ml 20X wash buffer in 570 ml of deionized water.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. If the concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the antibodies.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.
9. If crystals have formed in the Wash Solution concentrate (20X), warm to room temperature and mix gently until the crystals are completely dissolved.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at room temperature.

2. Pipette out **200 ul of Standards, Controls and Samples** into the respective wells.

3. Add **25 ul Biotin Conjugated Antibody** to all wells.

4. Add **25 ul of HRP conjugate** to each well.

5. Cover the plate and incubate for 120 minutes at room temperature on a shaker set at 170 rpm
6. Aspirate and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
7. Add **150 ul** of **TMB Substrate** to each well.
8. Cover the plate and incubate for 30 minutes at room temperature on a shaker set at 170 rpm
9. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
10. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Erythropoietin concentrations, find the unknown's Mean Absorbance value 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 Erythropoietin Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics:

Sensitivity:
The Lower Limit of Detection (LLD) is 1.2 mU/mL

Precision:
The Intra-assay precision of the EPO ELISA was calculated from 22 replicate determinations on each of the two samples

Intra-Assay Variation

Sample	Mean Value (mU/ml)	N	Coefficient of Variation %
A	14.4	22	8.4
B	189	22	4.8

The inter-assay precision of the EPO ELISA was calculated from data on two samples obtained in 22 different assays.

Inter-Assay Variation

Sample	Mean Value (mU/ml)	N	Coefficient of Variation %
A	20.4	22	8.8
B	183	22	5.1

Safety Precautions:

- **This kit is For Research Use Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

2. Components (2-8ºC) —30 mins RT Before Use→ 

Thaw at Room Temperature (18-24ºC) → USE NOW

Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

3.  Pipette **200 ul Standards, Controls and Samples** into respective wells.

4.  Add **25 ul Biotin Conjugated Antibody** to each well.

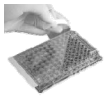
5.  Add **25 ul HRP Conjugate** to each well.

6. Cover plate  and incubate for 

at room temperature on a shaker at 170 rpm

7.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

8.  Pipette **100 ul TMB Substrate** into each well.

9. Cover plate  and incubate for 

at room temperature on a shaker at 170 rpm.

10. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	EPO equivalent
1A 2A	zero std zero std			
1B 2B	0 mU/ml 0 mU/ml			
1C 2C	10 mU/ml 10 mU/ml			
1D 2D	20 mU/ml 20 mU/ml			
1E 2E	40 mU/ml 40 mU/ml			
1F 2F	110 mU/ml 110 mU/ml			
1G 2G	Sample			
1H 2H	Sample			

LIMITED WARRANTY

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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