KRIBIOLISA™

EPO (Erythropoietin) ELISA

REF: KBBA01r

Ver1.1

RUO

ELISA for Accurate Quantitation of EPO from Rat Serum

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

KRISHGEN BioSystems Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400 018.
Tel: (022) 49198700 Email: sales@krishgen.com

Introduction:

Erythropoietin (EPO) is a heavily glycosylated protein with a molecular weight of about 30,000 - 34,000 Daltons. Rat 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:

The EPO ELISA is intended for the quantitative determination of Erythropoietin (EPO) in rat serum.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Samples and standards are pipetted into microwell precoated with anti-EPO antibody . After incubation and washing, anti-EPO antibody linked to Biotin is added to the wells. EPO present in the sample and standards will form a complex in the wells. HRP Conjugate is pipetted and incubated. Washing is done to remove the unbound HRP Conjugate. The ready to use substrate solution (TMB) is added to microwells and color develops directly proportional to the amount of EPO in the standards and sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-EPO antibody Coated Microtiter Plate (96 wells) – 1 no
2. Recombinant EPO Standard – 1 vial
3. Standard / Sample Diluent – 1 Vial.
4. Biotinylated EPO Antibody - 1 vial
5. HRP Conjugate - 1 vial
6. Wash Buffer (20X) – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate reader capable of reading at 450 nm and 405 nm.
2. Microplate washer.
3. Precision Pipettors to deliver 25, 200, 100 and 150 µl.
4. Timer.
5. Distilled or Deionized water.
6. Orbital rotator or shaker.
7. Linear graph Paper

Reagent Preparation and Storage:

1. Store all kit components at 2-8 °C.
2. All reagents except the Standards, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8 °C.
3. **Use the standards as soon as possible upon reconstitution.** ***Freeze (-15 °C) the remaining standards as soon as possible after use.*** Standards are stable at -15 °C for 6 weeks after reconstitution up to 3 freeze thaw cycles when handled as recommended in "Procedural Notes" section.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Procedural notes

1. Samples that have values below the limit of detection (50pg/ml) should be reported as "(50pg/ml)".
2. It is recommended that all Standards and samples are assayed in duplicates, until the analyst or technician has gained sufficient experience (as evidenced by the coefficient of variation duplicate being less than 10% [except for the values below the 2nd non-zero lowest standard] and the ability to obtain results for the kit controls within the suggested acceptable ranges).
3. The samples should be pipetted into the well with minimum amount of air-bubble. Samples with values greater than the highest Standard, which is approximately 3200pg/ml, must be diluted with Standard / Sample Diluent (Zero Standard) and re-assayed. Multiply the result by the dilution factor. Alternatively, the result may be reported as greater than the highest Standard concentration.
4. Reagents from different lot numbers must not be interchanged.
5. When mixing avoid splashing of reagents from wells. This will affect assay precision and accuracy.

Specimen Collection and Handling:

Serum:

Collect whole blood without anticoagulant and allow blood to clot between 2-8°C for 30 mins, if possible. It has been reported that serum samples clotted at room temperature (22°C to 28°C) caused a decrease in EPO value as assessed by radioimmunoassay of about 30% over clotting on ice. It is highly recommended that the specimen be collected between 7:30 a.m. to 12:00 p.m, because diurnal variation of erythropoietin has been reported in literature. Centrifuge the serum sample preferably in a refrigerated centrifuge for 15 mins. at 700 – 800 x g.

Store at - 20°C if the samples are not used on the same day. Avoid repeated freezing and thawing of the same sample.

Reagent Preparation:

1. Add 25 ml Wash Buffer (20X) to 475 ml of distilled water to make 1X working concentration. The diluted wash solution is stable for 90 days when stored at room temperature.

Assay Procedure:

1. Place sufficient **Coated Strips** in a holder to run Standards, Controls and samples.
2. Pipette 50 µl of Standard / Sample Diluent into the designated well.
3. Pipette 100 µl of Standards, controls and samples into the designated well.
4. Incubate for **2 hours** minutes at room temperature (22 -28°C).
5. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper.
6. Add or dispense **100 µl** of **Biotinylated EPO Antibody** into each of the wells and Incubate for **2 hour** at

room temperature (22 -28°C).

7. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper.
8. Add **100 µl** of **HRP Conjugate** into each of the same wells and Incubate for **1 hour** at room temperature (22 -28°C).
9. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper.
10. Add or dispense **100 µl** of the **TMB Substrate** into each of the wells and Incubate for **30min** at room temperature (22 -28°C).
11. Add or dispense **100 µl** of the **Stop Solution** into each of the wells.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to **450 nm with reference wavelength at 600-640nm.**

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 EPO 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 EPO Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.

- If the absorbance value is equivalent or higher than the 3200 pg/ml standard.

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 of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays. Please contact us for a copy of the validation guide for KRIBIOLISA™ EPO ELISA by email at sales@krishgen.com

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.
- 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

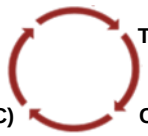
1. Remove all components, 15 minutes before adding into the assay plate.

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

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

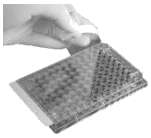
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

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

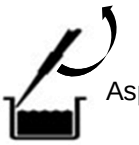


3.  Pipette **50 µl Standards** / **Samples diluent** into the respective wells

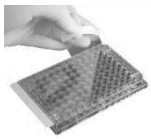
4.  Pipette **100 µl Standards** / **Samples** into the respective wells

5. Cover plate  and incubate 

at Room Temperature

6.  Aspirate and wash wells 5 times with **Wash Buffer (1X)**

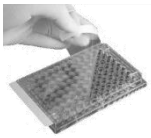
7.  Pipette **100 µl Biotynylated EPO Antibody** into each well

8. Cover plate  and incubate for 

at Room Temperature

9.  Aspirate and wash wells 5 times with **Wash Buffer (1X)**

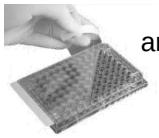
10.  Pipette **100 µl HRP conjugate** into each well

11. Cover plate  and incubate for 

at Room Temperature

12.  Aspirate and wash wells 5 times with **Wash Buffer (1X)**

13.  Pipette **100 µl TMB Substrate** into each well

14. Cover plate  and incubate for 

at Room Temperature

15.  Pipette **100 µl Stop Solution** into each well.

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

of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non- Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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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.

THANK YOU FOR USING KRISHGEN PRODUCT!