









KRIBIOLISA™ Anti-EPO (Erythropoietin) ELISA

REF: KBI6200

Ver1.0

RUO

ELISA for Accurate Quantitation of Anti-EPO from Human Serum and Plasma

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

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Introduction:

Erythropoietin (EPO) is a heavily glycosylated protein with a molecular weight of about 30,000 - 34,000 Daltons. Human 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 Anti- EPO ELISA is intended for the quantitative determination of Anti-Erythropoietin (EPO) in human serum and plasma.

Principle:

The method employs enzyme immunoassay technique. Samples and standards are pipetted into microwell pre-coated with EPO antigen. After incubation and washing, HRP Conjugate is added to the wells. Anti-EPO present in the sample and standards will form a complex in the wells. 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 Anti-EPO present in the standards and sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. EPO antigen Coated Microtiter Plate (96 wells) – 1 no
2. Anti- EPO Standard – 1 vial
3. Assay Diluent – 1 Vial
4. HRP Conjugate - 1 vial
5. Wash Buffer (20X) – 25 ml
6. TMB Substrate – 12 ml
7. Stop Solution – 12 ml
8. 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.

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 (10ng/ml) should be reported as "(10ng/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 640ng/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:

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. Bring all reagents to room temperature prior to use. It is strongly recommended that all Controls and Samples should be run in duplicates or triplicates. All steps must be performed at room temperature (RT).
2. Pipette **50 µl** of Assay Diluent into the designated well.

3. Pipette **100 µl** of Standards and samples into the designated well.
4. Incubate for **2 hours** 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 **100 µl** of **HRP Conjugate** into each of the same wells and Incubate for **1 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 or dispense **100 µl** of the **TMB Substrate** into each of the wells and Incubate for **30min** at room temperature (22 -28°C).
9. Add or dispense **100 µl** of the **Stop Solution** into each of the wells.
10. 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 Anti-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 Anti-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 640 ng/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™ Anti-EPO ELISA by email at sales@krishgen.com

Safety Instructions:

- **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.

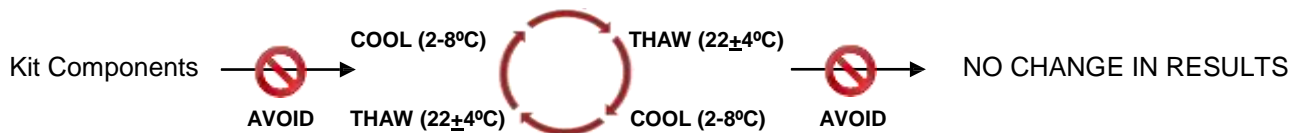


SCHEMATIC ASSAY PROCEDURE

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



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



3. Pipette **50 µl Assay Diluent** into each well.

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

5. Cover plate and incubate for at Room Temperature.

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

7. Pipette **100 µl HRP Conjugate** 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 TMB Substrate** into each well.

11. Cover plate and incubate for at Room Temperature.

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

13. 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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