KRISHGEN BioSystems

KRIBIOLISA™ EPO (Erythropoietin) ELISA

REF KBBA01

Ver 4.1

RUO

ELISA Set for Accurate Quantitation of Human Erythropoietin from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

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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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:

Erythropoietin (EPO) ELISA is specifically designed for the accurate quantitation of Erythropoietin (EPO) from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) - 1 no
2. Recombinant Human Erythropoietin Standard (100 IU/ml; lyophilized) - 1 vial
3. Biotinylated Anti-EPO Detection Antibody (lyophilized) - 1 vial
4. Streptavidin:HRP Conjugate – 1 vial
5. (20X) Wash Buffer - 25 ml
6. (1X) Assay Diluent - 50 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 able to measure absorbance at 450nm.
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.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant Standard and Detection Antibody at 2-8°C. Upon reconstitution, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

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.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.
Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot specific instructions for preparation of the reagents.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicates. A standard curve is required for each assay.

2. **Standards Preparation:** Reconstitute the lyophilized vial with 40 ul of Assay Diluent (1X) to generate a 100 IU/ml. Dilute 1 ul of original Standard (100 IU/ml) with 99 ul of Assay Diluent (1X) to generate a 1 IU/ml top standard. Perform serial dilutions by using top 200 mIU/ml top standard as per the below table. Thus, the Human Erythropoietin standard concentrations are 200 mIU/ml, 100 mIU/ml, 50 mIU/ml, 25 mIU/ml, 12.5 mIU/ml, 6.25 mIU/ml /ml and 3.13 mIU/ml. Assay Diluent (1X) serves as the zero standard.

Standard Concentration	Standard No	Dilution Particulars
100 IU/ml	Standard, lyophilized	Lyophilized Standard provided in the Kit + 40ul Assay Diluent (1X)
1 IU/ml	Mid Stock	10 ul Reconstituted Standard + 990 ul Assay Diluent (1X)
200 mIU/ml	Standard No.7	200 ul Mid Stock + 800 ul Assay Diluent (1X)
100 mIU/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent (1X)
50 mIU/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
25 mIU/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent (1X)
12.5 mIU/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
6.25 mIU/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
3.13 mIU/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
0 mIU/ml	Standard No.0	Only Assay Diluent (1X)

3. Bring all reagents to Room temperature before use. It is strongly recommended that all standards and Samples to be run in duplicates. A standard curve is required for each assay.

4. Add 100 ul of prepared **Standards and Samples** to the respective wells.

5. Seal the plate and incubate for 2 hours at Room Temperature (18-25°C).

6. Aspirate and wash plate 4 times with **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. All the washes should be performed similarly.

7. Add 100 ul of **Biotinlyated Anti-EPO Detection Antibody** solution to each well.

8. Seal the plate and incubate for 2 hours at Room Temperature (18-25°C).

9. Aspirate and wash plate 4 times with **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. All the washes should be performed similarly.

10. Add 100 ul of diluted **Streptavidin:HRP Conjugate** solution to each well. Seal plate.

11. Incubate for 30 minutes at Room Temperature (18-25°C).

12. Wash the plate 4 times with **Wash Buffer (1X)** as in Step 6**.**

13. Add 100 ul of **TMB Substrate** solution and incubate in the dark for 30 minutes at RT. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

14. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

15. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results:

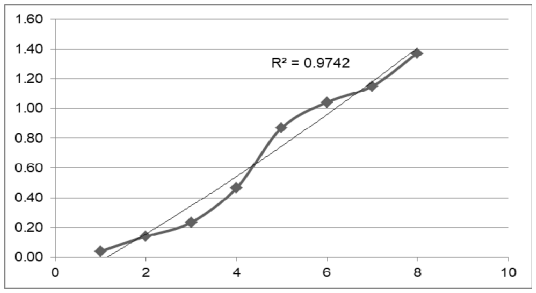
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on semi log graph paper, with the EPO concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown EPO concentrations, find the unknowns 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.

Computer based curve-fitting software like 4PL (2nd order) or cubic spline is preferred.

Typical Data

mIU/ml	Abs1	Abs2	Mean Abs
0	0.04	0.04	0.04
3.13	0.13	0.15	0.14
6.25	0.21	0.26	0.24
12.5	0.45	0.48	0.47
25	0.83	0.91	0.87
50	1.05	1.03	1.04
100	1.12	1.18	1.15
200	1.32	1.42	1.37

Typical Graph



Abs= absorbance at 450nm

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD. 10 replicates of '0' standards were evaluated and the LOD was found to **~2 mIU/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human Erythropoietin.

Cross Reactivity:
The following factors prepared at 100 ng/mL were assayed and exhibited no cross-reactivity or interference.
Recombinant human:
Albumin (prepared at 5 mg/mL)
IL-3
Thrombopoietin

Assay Range:
3.13 mIU/ml to 200 mIU/ml

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (6.25 mIU/ml), medium 25 mIU/ml) and high (100 mIU/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Sample	1:2	1:4	1:8
Serum (n=5)	84-107%	87-108%	82-112%
EDTA plasma (n=5)	83-102%	83-115%	83-118%
Heparin plasma (n=5)	83-99%	80-95%	82-93%

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.

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.

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THANK YOU FOR USING KRISHGEN PRODUCT

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SYMBOLS KEY

MTP	Anti-EPO Coated Microtiter Plate (12x8 wells)
STD	Erythropoietin Standard
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
1X ASY DIL	(1X) Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature