KRIBIOLISA® Erythromycin ELISA

REF : KRA1003

Ver 1.2

RUO

ELISA Set for Accurate Quantitative Analysis of Erythromycin residue in biological samples

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 KRA1003 Σ 96 tests

Krishgen Biosystems Private Limited
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.biz / www.krishgenbio.com

KRIBIOLISA® Erythromycin ELISA

Introduction:

Erythromycin residue in the production of biological products may lead to abnormal reactions of human beings, thus strict MRLs have been established. This kit is a rapid test product for the determination of tetracycline residues which is sensitive, accurate and time saving. It can considerably reduce the operation errors in the assay.

Intended Use:

This KRIBIOLISA® Erythromycin ELISA kit for Accurate quantitative and qualitative testing of Erythromycin residue in biological samples.

Principle:

This kit uses Indirect Competitive-ELISA as the method. It can detect Erythromycin residue in biological samples The microtiter wells are precoated with capture BSA-linked antigen. Samples and standards are pipetted into microwells and Erythromycin present in the sample will competes with antigen coated on the microtitre plate for the antibody. Then enzyme conjugate 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 inversely proportionally to the amount of Human Erythromycin in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated plate - 1x 96 wells
2. Standards (0, 0.2, 0.6 ,1.8, 5.4, 16.2 ng/ml) - 1 ml each
3. Spiking standard solution – (1 ml/bottle) - 1 ug/ml
4. Enzyme Conjugate - 7 ml
5. Antibody Solution - 7 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. (2X) Sample Diluent– 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution – 12 ml
10. Instruction Manual

Material required but not provided:

1. Microtiter plate spectrophotometer 450nm
2. Polystyrene centrifuge tubes: 2ml.
3. Micropipettes: 20ul-200ul, 200ul-1000ul, 250ul-multipipette
4. Deionized water

Storage Information:

1. Store main kit components at 2-8°C for 1 year. Avoid freeze / thaw cycles.
2. 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:

Dilute the sample with sample diluent (2X) for achieving proper Ampicillin concentration (0.2- 16.2 ng/ml) in it.

Reagent Preparation:

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. To make Wash Buffer (1X); Dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.
4. Sample Diluent (1X): Add 10 ml of Sample Diluent (2X) in 10 ml of DI water. Mix well

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. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Erythromycin.
3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. 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.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay procedure:

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

2. Add 50µl of standard solution or prepared sample to corresponding wells.

3. Add 50µl of detection antibody & enzyme conjugate into each well.

4. Mix gently by shaking the plate manually and incubate for 30 min at 25℃ with cover.

5. Aspirate and wash plate 4 times with 250 µl of Wash Buffer (1X) at interval of 10s and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off 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.

6. Add 100 µl of TMB Substrate to each well and incubate for 15 min at 25℃ with cover

7. Stop reaction by adding 100µl of Stop Solution to each well.

8. Read absorbance at 450nm within 30 minutes of stopping reaction. (It's suggested measure with the dual-wavelength of 450nm.

Calculation of Results:

1) Percentage absorbance:
The mean values of the absorbance values obtained from the standards and the samples are divided by the Absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance } (\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance of standards or samples
B0 ——absorbance of zero standard (0 ng/ml)

(2) Standard Curve:

1. To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the ampicillin standards solution (ppb) as x-axis.

2. The Ampicillin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

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.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.2 ng/ml.

Linearity:
Standards provided in the kit for graph range of 0.5 – 40.5 ng/ml

Precision: CV of the ELISA kit all less than 10%

Cross-Reactivity:

Erythromcin……..…...…100%
Erythromycin thiocyanate…….……..…114%
Ethylsuccinate ……..……...…67.4%
Tylosin.....<1%
Tilmicosin.....<1%
Spiramycin……..…….<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 at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide 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.

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