






KRIBIOLISA® Ampicillin ELISA

REF : KRA1002

Ver 1.1

RUO

ELISA Set for Accurate Quantitative And Qualitative Analysis of Ampicillin residue in vaccine

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 KRA1002  96 tests

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KRIBIOLISA® Ampicillin ELISA

Introduction:

Ampicillin belongs to the beta-lactams family, which once played very important role in preventing and curing animal diseases. For it causes anaphylactic reaction and resistance, in EU, US and China, it is being restricted. The common instrumental analysis of this drug is limited because of the complicated operation and high expense, while this kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 1.5h in single detection, so it can considerably minimize operation error and work intensity.

Intended Use:

This KRIBIOLISA® Ampicillin ELISA Kit for Accurate quantitative & qualitative analysis of Ampicillin residue in vaccine.

Principle:

KRIBIOLISA® Ampicillin ELISA kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with Ampicillin antigen. Ampicillin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the ampicillin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, ampicillin residue quantity in the sample can be calculated.

Materials Provided:

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Standards (1 ml/Vial) - 0, 0.2, 0.6, 1.8, 5.4, 16.2 ng/ml
3. Spiking standard solution (1 ml/Vial) – 1 ug/ml
4. Enzyme conjugate –12 ml
5. Antibody solution- 7ml
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

Materials to be provided by the End-User:

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
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.

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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:

Vaccine: Dilute the sample with the prepared (1X) Sample Diluent to get a final concentration of 0.2ng/ml - 16.2ng/ml.

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 Ampicillin.
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 **50 ul of Standard Solution** or **Prepared Sample** to corresponding wells.
3. Add **50 ul of Antibody Solution** into each well.
4. Mix gently by shaking the plate manually and incubate for 30 min at 25°C 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 ul of Enzyme Conjugate** in each well.
7. Mix gently by shaking the plate manually and incubate for **30 min** at **25°C** with cover.
8. Aspirate and wash as per step no. 5 above.
9. Add **100 ul of TMB Substrate** to each well and incubate for **15 min** at **25°C** with cover
10. Stop reaction by adding **100 ul of Stop Solution** to each well.

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

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

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. Please view the details herein below.

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 0.2 – 16.2 ng/ml

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



Cross-Reactivity:

Ampicillin	100%
Penicillin	52%
Penicillin V	< 20%

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.

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- 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 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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