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KRIBIOLISA™ Penicillin ELISA

REF : KRA1005

Ver 1.4

RUO

ELISA Set for Accurate Quantitation of Penicillin
for vaccine and biopharmaceutical use.

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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KRIBIOLISA™ PENICILLIN ELISA

Introduction:

Benzyl penicillin (BP) or Penicillin G is a broadly applied antibiotic, 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 1.5 hours in one detection, so it can considerably minimize operation error and work intensity.

Intended Use:

The KRIBIOLISA™ Penicillin ELISA Kit is intended for the accurate quantitation of Penicillin from biological samples including vaccines and biopharmaceuticals.

Principle:

KRIBIOLISA™ Penicillin ELISA kit is based on indirect-competitive ELISA. The microtiter wells are coated with Penicillin antigen. Penicillin residue in the sample competes with antigen coated on the microtitre plate for the antibody. After the addition of enzyme conjugate immune complex will form. After washing, addition of Substrate A and Substrate B is used to show the color. Absorbance of the sample is negatively related to the Penicillin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Penicillin residue in the sample can be calculated.

Materials Provided:

1. Penicillin coated Microtiter Plate (8 x 12 wells) - 1 no
2. Penicillin, Standards (0 ng/ml, 0.1 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml, 8.1 ng/ml) – 1 ml each
3. Spiking standard control: 1 ml, 1 ug/ml
4. Streptavidin:HRP Conjugate - 12 ml
5. Biotinylated Anti-Penicillin Detection Antibody - 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. Microplate Reader able to measure absorbance at 450 nm
2. Adjustable pipettes to measure volumes ranging from 5 ul to 1000 ul
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Polystyrene centrifuge tubes: 2 ml, 50 ml
7. Timers

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.

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 (1X) for achieving proper Penicillin concentration (0.1- 8.1 ng/ml) in it.

Reagent Preparation (all reagents should be diluted immediately prior to use):

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 Penicillin.
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 **Standards** or prepared **samples** to corresponding wells.

3. Add **50 ul** of **Biotinylated Anti-Penicillin Detection Antibody** into each well. Mix gently.

4. Incubate for **30 min** at **37^{o}C**.

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 Streptavidin:HRP Conjugate** in each well.

7. Incubate for **30 min** at **37^{o}C**.

8. Repeat Step No. (5)

9. Add **100 ul** of TMB Substrate to each well.

10. Incubate for **15 min** at **25°C**.

11. Stop reaction by adding **100 ul** of **Stop Solution** to each well.

12. 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%.

Absorbance (%) - B/B_0 X 100%

B = Absorbance of standards or samples
B_0 = Absorbance of zero standard (0 ng/ml)

(2) Standard Curve :

To draw a standard curve: The absorbance value of standards as y-axis, semi logarithmic of the concentration of the standards (ng/ml) as x-axis.

The Penicillin concentration of each sample (ng/ml), which can be read from the calibration curve, is multiplied by the corresponding dilution rate 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 0.08 ng/ml.

Linearity:
Standards provided in the kit for graph range of 0.1 - 8.1 ng/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, medium and high 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.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Cross-Reactivity:

Benzyl penicillin (BP).....100%
Ampicillin 0.7%
Cloxacillin 0.2%

Dicloxacillin.....0.1%
Amoxicillin .....<0.1%
Ceftiofur..... <0.1%

Safety Precautions:

- **This kit is for in vitro 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 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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