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Azithromycin ELISA Kit

02/21

(Catalog # E4945-100, 96 assays, Store at 4°C)

I. Introduction:

Azithromycin is a broad-spectrum antimicrobial drug that is used to treat respiratory, enteric, and genitourinary infections. It targets both gram-positive and gram-negative bacteria. The mode of action of this drug is that it binds to the 23S rRNA of the 50S ribosomal subunit of the bacteria. This prevents the transpeptidation/translocation step and thereby inhibits bacterial protein synthesis. Antibiotics such as Azithromycin have been used in food animals and aquaculture either to treat illnesses or as growth promoters. However, the use of Azithromycin in food animals may result in the formation of resistant bacteria and may lead to antibiotic resistance in humans. BioVision's Azithromycin ELISA kit is used to quantitatively measure Azithromycin in tissues, honey, and milk samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Azithromycin antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Azithromycin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Azithromycin
Detection Limit: 10ppb for raw milk, 4ppb for honey (Method 1) and 0.2ppb (Method 2), 15ppb for tissue
Sensitivity: 0.2ppb
Cross reaction: Azithromycin 100%, Erythromycin 100%

III. Sample Type:

Milk, Honey, Tissue

IV. Kit Contents:

Components	E4945-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4945-100-1
Standard (S0 – S5)	1 ml X 6	E4945-100-2
Enzyme Conjugate (11X)	0.7 ml	E4945-100-3
Enzyme Conjugate dilution	7 ml	E4945-100-4
Substrate A	7 ml	E4945-100-5
Substrate B	7 ml	E4945-100-6
Stop Solution	7 ml	E4945-100-7
Wash Buffer (20X)	30 ml	E4945-100-8
Redissolving solution	50 ml	E4945-100-9
Plate Sealer	3	E4945-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Trichloroacetic acid, NaOH, HCl, Na_2CO_3, $NaHCO_3$, Methanol, NaCl, Ethyl acetate
- Microplate reader capable of measuring absorbance at 450 nm, nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 1 part of **Wash buffer (20X)** with 19 parts of deionized water. Prepare quantity as needed.

2. **Enzyme Conjugate (1X):** Take 1 part of **Enzyme Conjugate (11X)** and add 10 parts of **Enzyme Conjugate diluent**. Mix well. Prepare fresh solution for the experiment.

3. **Standards Preparation:** Ready-to-use standards provided as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.2	0.6	1.8	5.4	16.2

4. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

FOR RESEARCH USE ONLY! Not to be used on humans.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Solution preparation before sample pre-treatment:

1) **2% NaCl solution:** Take 2 grams of NaCl and make up the volume to 100 ml using deionized water.
2) **Methanol: 2% NaCl solution:** Take 2 parts (100 ml) of Methanol and mix with 1 part (50 ml) of **2% NaCl solution**. Mix well.
3) **1% Trichloroacetic acid:** Take 5 grams of Trichloroacetic acid and make up the volume with 500 ml deionized water. Mix well.
4) **0.1M CB solution:** Take 4.66 grams of Na_2CO_3 and 0.5 grams of $NaHCO_3$ and make up the volume with 500 ml deionized water. Mix well.
5) **0.05M NaOH solution:** Take 1 gram of NaOH and make up the volume to 500 ml deionized water. Mix well.
6) **0.1M HCl solution:** Take 100 µl HCl and add 10.9 ml deionized water. Mix well.

Sample Preparation and pre-treatment (for milk samples):

Detection limit: 10ppb

- Take 1 ml of the milk sample into a 2 ml polystyrene centrifuge tube. Add 1 ml of **1% Trichloroacetic acid** solution. Vortex for 3 mins. Centrifuge at 3000 x g for 5 mins at room temperature (RT) (20 – 25 ^{0}C).
- Take 50 µl supernatant in a new 2 ml polystyrene centrifuge tube and add 450 µl **Redissolving solution**. Vortex for 1 min.
- Take 50 µl for analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for honey – Method 1):

Detection limit: 4ppb

- Take 1 ± 0.05 grams of the honey sample into a 50 ml polystyrene centrifuge tube. Add 2 ml of **Methanol: 2% NaCl solution**. Vortex for 2 mins until the honey is completely dissolved. Centrifuge at 3000 x g for 5 mins at room temperature (RT) (20 – 25 ^{0}C).
- Take 100 µl bright solution in a new 2 ml polystyrene centrifuge tube and add 900 µl **Redissolving solution**.
- Take 50 µl for analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for honey – Method 2):

Detection limit: 0.2ppb

- Take 2 ± 0.05 grams of the honey sample into a 50 ml polystyrene centrifuge tube. Add 2 ml of **0.1M CB solution**. Vortex until the honey is completely dissolved. Then add 6 ml **Ethyl acetate**, mix for 5 mins. Centrifuge at 3000 x g for 5 mins at room temperature (RT) (20 – 25 ^{0}C).
- Take 3 ml of the upper organic phase in a new 10 ml dry glass tube, blow-dry using nitrogen evaporator or water bath at 50 - 60 ^{0}C
- After blow-dry, add 0.5 ml **Redissolving solution** to the sample residue, Vortex for 5 mins.
- Take 50 µl for analysis.
- **Fold of dilution of the sample: 0.5**

Sample Preparation and pre-treatment (for tissue samples):

Detection limit: 0.2ppb

- Take 1 ± 0.05 grams of the tissue sample into a 50 ml polystyrene centrifuge tube. Add 4 ml of **0.05M NaOH solution**. Vortex for 5 mins. Centrifuge at 4000 x g for 5 mins at room temperature (RT) (20 – 25 ^{0}C).
- Take 200 µl of the upper clear liquid; add 0.75 ml **Redissolving solution** and 50 µl **0.1M HCl solution**. Vortex for 1 min (if the sample is cloudy, centrifuge the sample, take the upper clear layer and then analyze)
- Take 50 µl for analysis.
- **Fold of dilution of the sample: 25**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **Enzyme Conjugate (1X)** into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 minutes.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 250 µl of **Wash Buffer (1X)** to each well. Wash for 15-30 secs, and then discard the buffer. Repeat the washing step four to five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl of the **Substrate A**: **Substrate B mixture (prepare 1:1 mixture of Substrate A and Substrate B. The mixture must be used within 10 mins of preparation. Do not use metal container or metal to stir the solution)** into each well. Mix gently by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.

4. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for 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.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Azithromycin standards solution (ppb) as x-axis. The Azithromycin 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.

X. Related Products:

Streptomycin ELISA Kit (E4272)
Gentamicin (serum/urine) ELISA Kit (K4315)
Kanamycin ELISA Kit (K4210)
Quinolone ELISA Kit (E4530)
Fluoroquinolones ELISA Kit (K4205)