ab289843 – Azithromycin ELISA Kit

For the quantitative measurement of Azithromycin in tissue samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289843

Storage and Stability

On receipt unopened assay kit should be stored at 4°C for up to 12 months. Opened kit may be stable for 1 month at 4°C.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 wells	4°C
Standard (S0 – S5)	6 x 1 mL	4°C
Enzyme Conjugate	7 mL	4°C
Antibody working solution	7 mL	4°C
Substrate A	7 mL	4°C
Substrate B	7 mL	4°C
Stop Solution	7 mL	4°C
Wash Buffer (20X)	40 mL	4°C
Plate Sealer	1 unit	4°C
Spiking standard solution	1 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 10% Methanol
- Microplate reader capable of measuring absorbance at 450 nm, homogenizer, nitrogen-dying device, vortex, centrifuge, measuring pipets, and balance (a sensibility reciprocal of 0.01g), incubator
- Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 30-300 µL
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.
- Bring all reagents to room temperature 30 minutes before use.

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

Substrate A and Substrate B: Prepare a 1:1 mixture of Substrates A and B. Prepare a sufficient volume to provide 50µl per well. The mixture must be used within 10 mins of preparation, so perform this step just prior to use in the assay. Do not use a metal container or metal to stir the solution.

Standard Preparation:

- Ready-to-use standards are provided as follows:

Standards	S0	S1	S2	S3	S4	S5
Concentration	0	0.05	0.15	0.45	1.35	4.05

Sample Preparation:

Tissue: Place 1± 0.05 g of the homogenized fresh sample into a 50 mL centrifuge tube, add 5 mL of 10% methanol, then vortex for 3 min. Centrifuge at above 4000 r/min at room temperature (20-25°C) for 5 minutes. Take 50 µL of the supernatant for analysis.

Assay Protocol

- 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. Then add 50 µL of the Enzyme Conjugate (1X) and 50 µL of Antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate in the dark at 25°C for 30 minutes.
2. Remove the plate sealer carefully and aspirate the liquid out of the microwells. 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 the plate onto some absorbent paper to dry it. If there are the bubbles after tapping, remove them with clean tips.
3. Add 50 µL of the Substrate A : Substrate B mixture into each well. Mix gently by shaking the plate manually, and incubate at 25°C for 15 mins in the dark.
4. Add 50 µL of the Stop Solution into each well. Mix gently by shaking the plate manually. Set the test wavelength of the microplate reader to 450 nm and the reference wavelength to 630 nm, and read the OD value in each well within 5 mins.

Calculation:

The mean values of the absorbance 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%

Where
B = The average absorbance value of the sample or standard
B_0 = The average absorbance value of the 0 ppb standard

Standard curve: Plot the absorbance values on the y-axis, and the logarithmic of the concentration of Azithromycin (ppb) on the x-axis. Plot the axis within the range of the provided Standards' results. The Azithromycin concentration of each sample well (ppb) can be read from the calibration curve, and is then multiplied by the corresponding dilution factor of each sample to obtain the actual concentration in the sample.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

Version 1b, Last updated January 4, 2022