ab285232 – Doxycycline ELISA Kit

For the quantitative measurement of doxycycline in tissue, honey, milk, and serum.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285232

Storage and Stability

The entire ELISA 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.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 Strips	4°C
Standard 1 (0 ppb)	1 mL	4°C
Standard 2 (0.1 ppb)	1 mL	4°C
Standard 3 (0.3 ppb)	1 mL	4°C
Standard 4 (0.9 ppb)	1 mL	4°C
Standard 5 (2.7 ppb)	1 mL	4°C
Standard 6 (8.1 ppb)	1 mL	4°C
High Concentration Standard ()	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
20X Concentrated Washing Buffer	15 mL	4°C
20X Sample Extract A	15 mL	4°C
2X Sample Extract B	2 x 50 mL	4°C
20X Sample Diluent	10 mL	4°C

Materials Required, Not Supplied

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

- Equipment: Microplate reader, homogenizer, oscillator, centrifuge, balance, graduated pipette, incubator.
- Micropipettes: Single-channel 20~200 µL and 100~1000 µL, and multi-channel 30~300 µL.
- Reagents: NaOH, Acetonitrite.

Reagent Preparation

- 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.

Wash Buffer (1X): Dilute 15 mL of 20X Concentrated Washing Buffer with deionized water at 1:19 (1 part 20X Concentrated Washing Buffer + 19 parts deionized water). Alternatively, prepare washing buffer as quantity needed.

Sample Extract A: Dilute 1 part of 20X Sample Extract A + 19 parts of deionized water.

Sample Extract B: Dilutie 1 part of 2X Sample Extract B + 1 part of deionized water.

1M NaOH Solution: Weigh 4 g NaOH, add deionized water to 100 mL.

Sample Diluent: 1 part of 20X Sample Diluent + 19 parts of deionized water.

Standard Preparation

Standards	S1	S2	S3	S4	S5	S6	High Concentration Standard
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1	1000

Sample Preparation

- The prepared sample may be stored for up to one day at 4°C.
- **Sample pre-treatment:** The following method must be used for pre-treatment of any kind of sample: Note: Only disposable tips should be used for the experiments and the tips must be changed when used for different reagents.

Tissue, Honey

1) Homogenize 2g of tissue/honey sample into 3 mL diluted Sample Extract A and shake for 3 minutes.
2) Add 600 µL 1M NaOH solution and 2.4 mL diluted Sample Extract B. Shake for 3 minutes. Centrifuge at above 4000 r/min at room temperature for 5 minutes.
3) Take 50 µL up-layer clear liquid and add 450 µL diluted Sample Diluent. Mix the solution thoroughly.
4) Use 50 µL of the above mixed solution for analysis.
   Fold of dilution of the sample: 40

Serum

1) Add 1 mL serum sample into a 50 mL centrifuge tube. Add 1 mL of Acetonitrile and shake for 3 minutes.
2) Centrifuge at above 4000 r/min at room temperature for 5 minutes.
3) Take 100 µL up-layer clear liquid and add 400 µL diluted Sample Diluent. Mix the solution thoroughly.
4) Use 50 µL of the above mixed solution for analysis.
   Fold of dilution of the sample: 10

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay. Note that each liquid reagent must be shaken to mix evenly before use.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1) Add 50 µL of the sample or standard solution to separate duplicate wells. Add 50ul Enzyme conjugate and then 50 µL of the antibody solution into each well. Mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25°C for 30 minutes.
2) Pour liquid out of microwell. Add 250 µL/well of washing buffer for 15-30 seconds, repeat four to five times, and then flap to dry. If there are the bubbles after flapping, cut them with the clean tips.

3) Coloration: Add 50 µL of the Substrate A and then 50 µL of the Substrate B into each well. Mix gently by shaking the plate manually. Incubate at 25°C for 15 minutes in the dark for coloration.
4) Determination: 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 to read the OD value at the dual-wavelength 450/630 nm within 5 minutes).

Calculations

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%

Where: **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: Take the absorbency value of standards as y-axis, logarithm of the Doxycycline standards concentration (ppb) as x-axis. The Doxycycline 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.

Qualitative Determination: The concentration range (ng/mL) can be obtained by comparing the average OD value of the sample with that of the standard solution.

- Example: Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, while those of the standard solutions are as the followings: 2.243 for 0ppb, 1.816 for 0.1ppb, 1.415 for 0.3ppb, 0.74 for 0.9ppb, 0.313 for 2.7ppb and 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1ppb, and that of the sample Ⅱ is 0.3 to 0.9ppb.

Technical Support

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