ab285226 – Streptomycin ELISA Kit

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

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285226

Storage and Stability

The entire kit may be stored at 4°C for 12 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	1	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 Standard (1000 ppb)	1 ml	4°C
Antibody Working Solution	5.5 ml	4°C
Enzyme Conjugate	11 ml	4°C
Substrate A solution	6 ml	4°C
Substrate B solution	6 ml	4°C
Stop Solution	6 ml	4°C
Concentrated Wash Solution (20X)	40 ml	4°C
Concentrated Redissolving Solution (5X)	50 ml	4°C
Plate sealer	1	4°C

Δ Note: High Standard (1000 ppb) included is for recovery experiments

Materials Required, Not Supplied

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

- Reagents: NaOH, Na2HPO4•12H2O, NaH2PO4•2H2O, N-hexane, Dichloromethane, Acetonitrile, H3PO4.
- Microplate reader capable of measuring absorbance of 450 nm
- Precision pipettes with disposable tips
- Distilled or deionised water
- Clean Eppendorf tubes for preparing standards and sample dilutions
- Absorbent paper

Reagent Preparation

Δ Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

PB buffer: Dissolve 12.9 g Na2HPO4•12H2O and 2.175 g NaH2PO4•2H2O into deionized water to 100 ml.
Redissolving solution: Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4 ℃ environment up to a month.

Wash Buffer: Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.

Standard Preparation

Ready to use:

Tube	S1	S2	S3	S4	S5	S6
Concentration (ng/ml)	0	0.1	0.3	0.9	2.7	8.1

Sample Preparation

Δ Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

Tissue: Weigh 2 g Homogeneous sample into a centrifuge tube, add 8 ml PB buffer, oscillate 5min, and incubate for 30 min at water bath at 56°C. Centrifuge at 4000 r/min at room temperature for 10 min. Take 1ml supernatant to another centrifuge tube, add 1 ml Nhexane, mix fully, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper N-hexane, take 50 µl Lower water phase, add 450 µl redissolving solution, and mix 30s. Use 50µl for the assay. (Dilution times of the sample: 1:40)

Honey: Weigh 2 g honey sample into centrifuge tube. Add 4 ml 0.04 M H3PO4, oscillation until completely dissolved, centrifuge at 4000 rpm at room temperature for 5 min until clear. Add 450 µl 1M NaOH, adjust to pH 7-9, centrifuge at 4000 rpm at room temperature for 5 min until clear. Take 50 µl supernatant, add 450 µl redissolving solution, and mix 30 seconds. Use 50 µl for the assay. (Dilution times of the sample: 1:20)

Milk, Milk powder: Weigh 2 g samples into centrifuge tube, add 8 ml PB buffer, oscillate 5 min, and incubate for 30 min at water bath environment at 56°C. Centrifuge at 4000 rpm at room temperature for 10 min. Wipe out the upper fat, take 50 µl middle clarified liquid, add 450 µl redissolving solution, and mix 30 sec. Use 50µl for the assay. (Dilution times of the sample: 1:50)

Assay Protocol

Δ 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) Prepare all reagents, samples and standards as instructed.
2) Add 50 µl standards or samples into marked well. Add 50 µl antibody working solution into each well.
3) Oscillate the plate for 5 sec, cover the well and incubate for 30 min at RT (25°C).
4) Discard solution, wash plate 5 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5) Add 100 µl Enzyme conjugate into each well; avoid the light to incubate for 30 min at 25°C.
6) Repeat washing procedure to step 4.
7) Pipette 50 µl Substrate A solution, then pipette 50 µl Substrate B solution to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT.
8) Add 50 µl Stop Solution to each well and oscillate gently to stop the reaction.

Measurement
Read absorbance at 450 nm within 10 minutes of adding stop solution.

Calculation:

Percentage of absorbance value (%) = A/A_0 x 100%

A: the average (double wells) OD value of the sample or the standard solution

A_0: the average OD value of the 0 ppb standard solution.

- To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semi log curves with X-axis and Y-axis.
- Plot the absorbance percentage of samples into the standard curve, to get the concentration for each sample and multiply by the dilution to give the concentration of streptomycin in samples.

Technical Support

For all technical or commercial enquiries please go to:
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