Streptomycin (SM) ELISA Kit

Catalog No.: abx364836

Size: 96 tests

Detection Limit: Muscle – 4 ng/ml (ppb); Honey – 2 ng/ml (ppb); Milk, Milk powder – 5 ng/ml (ppb); Egg – 10 ng/ml (ppb).

Cross-Reactivity: Streptomycin, Dihydrostreptomycin – 100%; Clarithromycin – 6.3%; Gentamicin – 2.5%.

Storage: Store all components at 4°C.

Application: For quantitative detection of Streptomycin concentration in muscle tissue, honey, milk, milk powder, and egg.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Streptomycin is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to Streptomycin are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain Streptomycin will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Streptomycin amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Streptomycin can be calculated.

Kit components

1. 96-well microplate
2. Standard vials (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. Wash Buffer (20X): 40 ml
4. Detection Reagent A: 5.5 ml
5. Detection Reagent B: 11 ml
6. Reconstitution Buffer (5X): 50 ml
7. Substrate Reagent A: 6 ml
8. Substrate Reagent B: 6 ml
9. Stop Solution: 6 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials required but not provided

1. Microplate reader (450 nm)
2. Pipette and pipette tips
3. Microcentrifuge tubes
4. Centrifuge
5. Vortex mixer
6. Water bath
7. Incubator

Reagents required but not provided

1. Double-distilled water
2. Solid (powder) sodium hydroxide (NaOH)
3. Solid hydrated disodium phosphate ($Na_2HPO_4•12H_2O$)
4. Solid hydrated sodium dihydrogen phosphate ($NaH_2PO_4•2H_2O$)
5. N-Hexane
6. 85% Phosphoric acid (H_3PO_4)
7. Methanol
8. 99.5% Ethanoic acid (CH_3COOH)

Protocol

A. Preparation of samples and reagents

1. Reagents

- **0.05 M Phosphate Buffer:** Carefully weigh and dissolve 12.9 g of hydrated disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$) in 1000 ml of double-distilled water, followed by 2.175 g of hydrated sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$). Mix thoroughly to ensure all reagents have fully dissolved. *For use with muscle tissue, milk, and milk powder samples.*
- **0.04 M Phosphoric Acid Solution:** Add 1 ml of 85% Phosphoric acid (H_3PO_4) to 359 ml double-distilled water. Mix fully. *For use with honey samples.*
- **1 M Sodium Hydroxide Solution:** Dissolve 4 g of solid sodium hydroxide (NaOH) in 100 ml double-distilled water. Mix thoroughly to ensure all reagents have fully dissolved. *For use with honey samples.*
- **1% Ethanoic Acid Solution:** Add 1 ml 99.5% Ethanoic Acid Solution to 99 ml double-distilled water. Mix fully. *For use with egg samples.*
- **70% Methanol Solution:** Add 700 ml of Methanol to 300 ml double-distilled water. Mix fully. *For use with egg samples.*
- **Reconstitution Buffer (1X):** Dilute the Reconstitution Buffer (5X) with double-distilled water in a ratio of 1:4 (i.e. for 50 ml Reconstitution Buffer (5X), add 200 ml double-distilled water).
- **Wash Buffer (1X):** Dilute the Wash Buffer (20X) with double-distilled water in a ratio of 1:19 (i.e. for 25 ml of Wash Buffer (20X), add 475 ml of double-distilled water).

Note:

- Allow all reagents to equilibrate to room temperature before preparation.
- Prepare these solutions according to the number of wells that are going to be tested, using appropriate volumes of the stock reagents.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Muscle tissue:** Remove fat from sample and homogenize manually – avoid lysis buffers. Carefully weigh out 2 ± 0.05 g of homogenate, and add into a centrifuge tube. Add 8 ml of 0.05 M Phosphate Buffer and vortex for 5 minutes. Incubate in a water bath for 30 minutes at 56°C. Centrifuge at 4000 RPM for 5 minutes at room temperature, then take 1 ml of supernatant into a fresh centrifuge tube. Add 1 ml of N-Hexane and mix fully by inverting the tube, then centrifuge at 4000 RPM for 5 minutes at room temperature. Discard the uppermost liquid layer, and take 50 µl of the lower layer into a fresh centrifuge tube. Add 450 µl of Reconstitution Buffer (1X), and mix fully for 30 seconds. Take 50 µl of the resulting solution for analysis.

Note: Sample dilution factor: 40; Detection limit: 4 ng/ml.

- **Honey:** Carefully weigh 2 ± 0.05 g of sample into a centrifuge tube. Add 4 ml of 0.04 M Phosphoric Acid Solution and vortex the tube until the sample has dissolved fully. Centrifuge at 4000 RPM for 5 minutes. Transfer the supernatant to a fresh centrifuge tube, and add 450 µl of 1 M Sodium Hydroxide Solution to neutralize the sample to pH 7-9. Centrifuge at 4000 RPM for 5 minutes. Take 50 µl of supernatant into a fresh centrifuge tube, and add 450 µl of Reconstitution Buffer (1X). Mix fully for 30 seconds. Take 50 µl for analysis.

Note: Sample dilution factor: 20; Detection limit: 2 ng/ml.

- **Milk** and **Milk powder:** Carefully weigh 2 ± 0.05 g of sample into a centrifuge tube. Add 8 ml of 0.05 M Phosphate Buffer and vortex for 5 minutes. Incubate in a water bath for 30 minutes at 56°C, and then centrifuge at 4000 RPM for 10 minutes at room temperature. Discard the uppermost lipid layer, and take 50 µl of the middle layer into a fresh centrifuge tube. This layer should be clear. Add 450 µl of Reconstitution Buffer (1X), and mix thoroughly for 30 seconds. Take 50 µl for analysis.

Note: Sample dilution factor: 50; Detection limit: 5 ng/ml.

- **Egg:** Remove all shell from the sample, and homogenize the yolk and white together manually – avoid lysis buffer. Carefully weigh 1 ± 0.05 g of homogenate into a centrifuge tube. Add 2 ml of 1% Ethanoic Acid Solution, and vortex for 2 minutes. Add 7 ml of 70% Methanol Solution, and vortex for 2 minutes. Centrifuge at 4000 RPM for 10 minutes. Take 100 µl of supernatant into a fresh 1.5 ml centrifuge tube, and add 900 µl of Reconstitution Buffer (1X). Mix fully for 30 seconds. Take 50 µl for analysis.

Note: Sample dilution factor: 100; Detection limit: 10 ng/ml.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods.
- Sample dilution factors are provided for reference only. Actual sample concentrations may vary, and a series of test dilutions must be performed before running a full assay to determine the optimal dilution factor for these specific samples.

B. Assay Procedure

1. Mark well positions for each standard and sample. *It is strongly recommended to test all wells in duplicate.*
2. Add 50 µl of each sample to the sample wells.
3. Add 50 µl of each standard concentration to the corresponding standard wells.
4. Add 50 µl of Detection Reagent A to all wells.
5. Seal with a plate sealer, and tap the plate gently for 5 seconds to mix the well contents.
6. Incubate for 30 minutes at 25°C in the dark.
7. Remove the cover and discard the solution. Wash the plate 5 times with Wash Buffer(1X). Do this by filling each well with 300 µl Wash Buffer (1X) using a multi-channel pipette and leaving it to soak for 30 seconds, before removing and then refreshing the buffer.
8. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels. *Complete removal of liquid at each step is essential for good performance.*
9. Add 100 µl of Detection Reagent B to all wells.
10. Seal with a plate sealer, and incubate for 30 minutes at 25°C in the dark.

11. Remove the cover and discard the solution. Wash the plate 5 times with Wash Buffer(1X). Do this by filling each well with 300 µl Wash Buffer(1X) using a multi-channel pipette and leaving it to soak for 30 seconds, before removing and then refreshing the buffer.
12. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels. *Complete removal of liquid at each step is essential for good performance.*
13. Add 50 µl of Substrate Reagent A to all wells.
14. Add 50 µl of Substrate Reagent B to all wells.
15. Seal with a plate sealer, and tap the plate gently for 5 seconds to mix the well contents.
16. Incubate for approximately 15 minutes at 25°C. *The final incubation time can be adjusted depending on the progress of the color development.*
17. Add 50 µl of Stop Solution to each well. Tap the plate gently to mix the well contents.
18. Immediately measure the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

This assay is competitive, therefore there is an inverse correlation between Sulfamerazine concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

$$\%\ \text{Absorbance} = \frac{A}{A_0} \times 100$$

where:

A	Average absorbance of the sample well
A_0	Average absorbance of the blank (0 ng/ml) well

Plot the Absorbance (%) on the y-axis, and the log concentration (ng/ml) on the x axis. Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

D. Precautions

1 Bring all reagents to room temperature prior to use.
2 Do not allow the wells to dry fully during the washing procedure.
3 Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
4 Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
5 Do not use any reagents that are expired, or use reagents from other kits with this assay.
6 The Stop Solution is corrosive. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
7 It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.