Spectinomycin (SM) ELISA Kit

Catalog No.: abx365095

Size: 96 tests

Detection Limit: Muscle, Milk, Egg – 50 ng/ml (ppb).

Cross-Reactivity: Spectinomycin – 100%,
Streptomycin, Neomycin, Kanamycin, Gentamicin, Dihydrostreptomycin - < 0.01%

Storage: Store all components at 2-8°C.

Application: For quantitative detection of Spectinomycin concentration in muscle tissue, milk, and egg.

Introduction

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Spectinomycin is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to Spectinomycin 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 Spectinomycin 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 Spectinomycin amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Spectinomycin can be calculated.

Kit components

1. 96-well microplate
2. Standards (0 ng/ml, 0.5 ng/ml, 1 ng/ml, 2 ng/ml, 4 ng/ml, 8 ng/ml): 1 ml each
3. Detection Reagent A: 7 ml
4. Detection Reagent B: 7 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Sample Diluent (20X): 15 ml
9. Wash Buffer (20X): 25 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials required but not provided

1. Microplate reader (450 nm)
2. Balance (0.01 g)
3. Deionized water
4. Trichloroacetic acid
5. Pipette and pipette tips
6. Microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

Allow all reagents to equilibrate to room temperature before preparation. Experimental apparatus should be clean and the pipettes should be disposable to avoid cross-contamination. Prepare the solution according to the number of samples.

- **2.5% Trichloroacetic Acid Solution:** Dissolve 2.5 g of Trichloroacetic Acid in 100 ml of deionized water. Mix fully.
- **1X Sample Diluent:** Dilute Sample Diluent (20X) with deionized water in a 1:19 ratio.
- **1X Wash Buffer:** Dilute Wash Buffer (20X) with deionized water in a 1:19 ratio.

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, milk, and egg:** Remove fat from sample and homogenize manually – avoid using lysis buffers. Carefully weigh out 1 g of tissue, add 1 ml of 2.5% Trichloroacetic Acid Solution and vortex for 1 minute. Centrifuge at 4000 rpm for 5 minutes. Take 20 µl of intermediate liquid into a fresh 2 ml centrifuge tube. Add 980 µl of 1X Sample Diluent and vortex for 30 seconds. Mix fully. Take 20 µl of the supernatant for analysis.

  Note: Sample dilution factor: 100, Detection limit: 50 ng/ml

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- The sample dilution factors are for reference only. Actual sample concentrations may vary, and a series of test dilutions must be performed to determine the optimal dilution factor for these specific samples.

B. Assay Procedure

Bring all reagents and samples to room temperature before use. All reagents should be mixed thoroughly before pipetting. Avoid foaming.

1. Set standard and sample wells on the 96 well microplate and label accordingly. *It is recommended to test standards and samples in duplicate.*
2. Add 20 µl of each sample to each sample well.
3. Add 20 µl of each standard to each standard well.
4. Add 50 µl of Detection Reagent B to all wells.
5. Add 50 µl of Detection Reagent A to all wells.
6. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
7. Seal with a plate sealer, and incubate for 30 minutes in the dark at 25°C.
8. Remove the cover and discard the solution. Wash the plate 4 times with 1X Wash Buffer. Do this by filling each well with 260 µl 1X Wash Buffer using a multi-channel pipette and leaving it to soak for 30 seconds, before removing and then refreshing the buffer.
9. After the final wash, remove any remaining 1X 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.*
10. Add 50 µl of Substrate Reagent A to all wells.
11. Add 50 µl of Substrate Reagent B to all wells.
12. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
13. Seal with a plate sealer, and incubate for approximately 10-20 minutes in the dark at 25°C.
14. Add 50 µl of Stop Solution to each well. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
15. 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 Spectinomycin concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

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

A	Average absorbance of standard/sample
A_0	Average absorbance of 0 ng/ml standard

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. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
3. Do not allow the wells to dry fully during the washing procedure.
4. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
5. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
6. Do not use any reagents that are expired, or use reagents from other kits with this assay.
7. The Stop Solution is corrosive. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
8. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.
9. This kit is for research use only.