Gentamicin (Gen) ELISA Kit

Catalog No.: abx365005

Size: 96 tests

Detection Limit: Muscle – 10 ng/ml (ppb); Egg – 10 ng/ml (ppb); Milk – 15 ng/ml (ppb).

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

Storage: Store all components at 4°C.

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

Introduction

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

Kit components

1. 96-well microplate
2. Standard vials (0 ng/ml, 0.1 ng/ml, 0.2 ng/ml, 0.6 ng/ml, 1.8 ng/ml, 5.4 ng/ml): 1 ml each
3. Wash Buffer (20X): 25 ml
4. Sample Solution (4X): 25 ml
5. Tissue Extraction Solution (10X): 100 ml
6. Substrate Reagent A: 6 ml
7. Substrate Reagent B: 6 ml
8. Detection Reagent A: 7 ml
9. Detection Reagent B: 7 ml
10. Stop Solution: 6 ml
11. Plate sealer: 3
12. Hermetic bag: 1

Materials required but not provided

1. Microplate reader (450 nm)
2. Distilled water
3. Solid (powder) Trichloroacetic acid
4. Solid (powder) NaCl
5. Pipette and pipette tips
6. Microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Wash Buffer (1X):** Dilute the Wash Buffer (20X) with distilled water in a ratio of 1:19 (i.e. for 25 ml of Wash Buffer (20X), add 475 ml of distilled water).
- **1% Trichloroacetic Acid Solution:** Dissolve 1 g of Trichloroacetic Acid powder in 100 ml of distilled water. Mix fully. *For use with milk samples.*
- **Working Sample Solution (1X):** Dilute the Sample Solution (4X) with distilled water in a ratio of 1:3 (i.e. to 25 ml of Sample Solution (4X), add 75 ml distilled water). *For use with milk samples.*
- **Working Tissue Extraction Solution (1X):** Dilute 100 ml of Tissue Extraction Solution (10X) with 900 ml distilled water. Add 20 g of NaCl, and mix fully to ensure it has completely dissolved. *For use with muscle tissue and egg samples.*

Note:

- Allow all reagents to equilibrate to room temperature before preparation.

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, egg:** Remove fat from sample and homogenize manually – avoid using lysis buffers. Carefully weigh out 1 g of tissue, and add into a 50 ml centrifuge tube. Add 10 ml of Working Tissue Extraction Solution (1X). Vortex for 1 minute, then centrifuge at 4000 rpm for 5 minutes at room temperature. Take 50 µl of the supernatant for analysis.

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

- **Milk:** Add 1 g of sample to 1 ml of 1% Trichloroacetic Acid Solution in a 5 ml centrifuge tube and vortex for 1 minute. Centrifuge at 4000 rpm for 5 minutes at room temperature. Take 50 µl of the supernatant into a 2 ml centrifuge tube. Add 950 µl of Working Sample Solution (1X) and vortex for 30 seconds to mix fully. Take 50 µl for analysis.

  Note: Sample dilution factor: 40; Detection limit: 15 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 for cell lysates and tissue homogenates.
- 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

1. Mark well positions for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 50 µl of each sample to each respective sample well.
3. Add 50 µl of each standard to its respective standard well.
4. Add 50 µl of Detection Reagent A to all wells.
5. Add 50 µl of Detection Reagent B 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 at 25°C.
8. Remove the cover and discard the solution. Wash the plate 4 times with Wash Buffer (1X). Do this by filling each well with 260 µl Wash Buffer (1X) 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 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 15 minutes at 25°C. *The final incubation time can be adjusted depending on the progress of the color development.*
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 TCs concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

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

where:

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.