Tylosin ELISA Kit

Catalog No.: abx364996

Size: 96 tests

Detection Limit: Muscle – 1 ng/ml (ppb); Honey – 0.5 ng/ml (ppb), Egg, Milk – 25 ng/ml (ppb).

Cross-Reactivity: Tylosin – 100%; Erythromycin – 1%; other macrolide antibiotics - < 1%.

Storage: Store all components at 4°C.

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

Introduction

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

Kit components

1. 96-well microplate
2. Standard vials (0 ng/ml, 0.5 ng/ml, 1.5 ng/ml, 4.5 ng/ml, 13.5 ng/ml, 40.5 ng/ml): 1 ml each
3. Wash Buffer (20X): 40 ml
4. Reconstitution Buffer (2X): 50 ml
5. Detection Reagent A: 5.5 ml
6. Detection Reagent B: 11 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. Distilled water
3. Pipette and pipette tips
4. Microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

Reagents required but not provided

1. Sodium Hydroxide
2. Methanol
3. Hydrochloric acid (0.1 M)
4. Acetonitrile
5. Trichloromethane
6. N-hexane

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **0.1 M Sodium Hydroxide:** Dissolve 0.4 g of Sodium Hydroxide in 100 ml distilled water and mix fully.
- **0.01 Sodium Hydroxide**: Dissolve 0.4 g of Sodium Hydroxide in 1000 ml distilled water and mix fully.
- **Extraction Solution:** Mix 84 ml of Acetonitrile with 16 ml of 0.1 M Hydrochloric acid and 18 ml of Methanol. Mix fully.
- **Reconstitution Buffer (1X):** Dilute the 2X Reconstitution Buffer 2-fold with distilled water and mix fully.
- **Wash Buffer (1X):** Dilute the Wash Buffer (20X) with distilled water in a ratio of 1:19 (i.e. for 40 ml of Wash Buffer (20X), add 760 ml of distilled water).

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:** Remove fat from sample and homogenize manually – avoid using lysis buffers. Carefully weigh out 2 g of tissue, and add into a 50 ml centrifuge tube. Add 9 ml of Extraction Solution and vortex for 5 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 2 ml of the supernatant, add 1 ml of 0.1 M Sodium Hydroxide and mix fully. Add 3 ml Trichloromethane and vortex for 5 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Discard the upper layer supernatant, take the lower organic supernatant to a glass centrifuge tube. Dry at 56°C using a nitrogen evaporator or a water bath in a ventilated environment. Dissolve the solid residue with 1 ml of 1X Reconstitution Buffer, vortex for 1 minute to mix fully. Take 50 µl of the supernatant for detection.

  Note: Sample dilution factor: 2; Detection Limit: 1 ng/ml

- **Milk:** Add 0.5 ml of milk sample to 2 ml of 0.01 M Sodium Hydroxide solution and vortex for 2 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 1 ml of supernatant, add 2 ml of n-hexane and vortex for 2 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Discard the upper n-hexane layer, and take 0.1 ml of the lower layer to a new tube. Add 0.9 ml of 1X Reconstitution Buffer, then vortex for 1 minute to mix fully. Take 50 µl for analysis.

  Note: Sample dilution factor: 50; Detection Limit: 25 ng/ml.

- **Honey:** Carefully weigh 1 g of sample into a 50 ml centrifuge tube. Add 2 ml of distilled water and vortex thoroughly for 2 minutes. Add 10 ml of Trichloromethane, vortex for 5 minutes, then centrifuge at 4000 rpm for 10 minutes at room temperature. Discard the upper layer supernatant, take the lower organic supernatant to a glass centrifuge tube. Dry at 56°C using a nitrogen evaporator or a water bath in a ventilated environment. Dissolve the solid residue with 1 ml of 1X Reconstitution Buffer, vortex for 1 minute to mix fully. Take 50 µl of the supernatant for detection.

  Note: Sample dilution factor: 1; Detection Limit: 0.5 ng/ml.

- **Egg:** Remove any skin from the sample, and homogenize with a homogenizer. Weigh 1 g of homogenized sample, and add 4 ml of 0.01 M Sodium Hydroxide solution and vortex for 2 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 2 ml of supernatant, add 4 ml of n-hexane and vortex for 2 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Discard the upper n-hexane layer, and take 0.1 ml of the lower layer to a new tube. Add 0.9 ml of 1X Reconstitution Buffer, then vortex for 1 minute to mix fully. Take 50 µl for analysis.

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Note: Sample dilution factor: 50; Detection Limit: 25 ng/ml.

Notes:

- 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 provided 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. Set standard and sample wells on the 96 well microplate and label accordingly. *It is 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. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
6. Seal with a plate sealer, and incubate for 30 minutes at 25°C.
7. Remove the cover and discard the solution. Wash the plate 5 times with Diluted Wash Buffer (1X). *Do this by filling each well with 260 µl Diluted Wash Buffer (1X) using a multi-channel pipette and leaving it to soak for 30 seconds, before removing and then refreshing the buffer. 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.*
8. Add 100 µl of Detection Reagent B to all wells.
9. Remove the cover and discard the solution. Wash the plate 5 times as described
10. Seal with a plate sealer, and incubate for 30 minutes at 25°C.
11. Wash the plate 5 times as described in step 7.
12. Add 50 µl of Substrate Reagent A to all wells.
13. Add 50 µl of Substrate Reagent B to all wells.
14. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
15. Seal with a plate sealer, and incubate for approximately 10-20 minutes at 25°C.
16. Add 50 µl of Stop Solution to each well. Shake the plate gently for 10 seconds to mix the well contents thoroughly.
17. Immediately measure the OD of each well with a microplate reader at 450 nm.

C. Calculations

This assay is competitive, therefore there is an inverse correlation between Tylosin 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.

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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.

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