Ivermectin (IVM) ELISA Kit

Catalog No.: abx365136

Size: 96T

Storage: Store at 4°C.

Application: The quantitative detection of Ivermectin in milk and yogurt.

Detection Limit: 1 ng/ml

Principle of the assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the the pre-coated SAs and the SAs in the sample with the biotin-labelled antibody. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient IVM will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the color yellow is inversely proportional to the Ivermectin amount bound on the plate. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of SAs can be calculated.

Kit components

1. Pre-coated 96-well microplate
2. Standards (0.8 ng/ml, 0.4 ng/ml, 0.2 ng/ml, 0.1 ng/ml, 0.05 ng/ml, 0 ng/ml): 1 ml each
3. Wash Buffer: (20X) 25 ml
4. Milk Precipitant: 10 ml
5. Sample Diluent A: 20 ml
6. Sample Diluent B: 20 ml
7. TMB Substrate A: 6 ml
8. TMB Substrate B: 6 ml
9. Detection Reagent A: 7 ml
10. Detection Reagent B: 12 ml
11. Stop solution: 6 ml
12. Hermetic bag: 1
13. Plate sealer: 1

Materials required but not provided

1. Microplate reader (450 nm)
2. Single-channel and multi-channel pipette and pipette tips
3. Centrifuge
4. Vortex mixer
5. Balance
6. Incubator
7. Centrifuge tubes
8. Deionized water
9. Methanol anhydrous

Protocol

A. Preparation of samples and reagents

1. Reagent preparation

- **1X Wash Buffer:** Make up sufficient amounts for the wells tested. Dilute 20X Wash Buffer 20 fold with deionized water (for example add 1 ml of 20X Wash Buffer to 19 ml of deionized water to make 20 ml of 1X Wash Buffer).

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Overall OD may be lower if reagents are below 25°C.
- Substrate reagent should not be used if it appears blue.

2. Sample pretreatment

- **Milk (raw and pasteurized):** Add 2 ml of sample to a 10 ml centrifuge tube. Add 100 µl of Milk Precipitant and 3 ml of methanol anhydrous. Vortex for 1 minute to mix thoroughly. Centrifuge at 4000 RPM for 5 minutes. Carefully collect 200 µl of the supernatant and add to a new tube. Add 200 µl of Sample Diluent A and mix thoroughly for 30 seconds. Aliquot 50 µl and assay immediately.
  Note: Sample dilution factor 5, Detection limit: 1 ng/ml.

- **Yogurt:** Add 2 ml of sample to a 10 ml centrifuge tube. Add 3 ml of methanol anhydrous and vortex for 1 minute to mix thoroughly. Centrifuge at 4000 RPM for 5 minutes at room temperature. Carefully collect 200 µl of the supernatant and add to a new tube. Add 200 µl of Sample Diluent B and mix thoroughly for 30 seconds.
  Note: Sample dilution factor 5, Detection limit: 1 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 tissue homogenates.
- Allow samples to equilibriate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark the positions of each well for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 50 µl of sample to each sample well.
3. Add 50 µl of standard to each standard well.
4. Add 50 µl of Detection Reagent A to each well.
5. Tap the plate gently for 10 seconds to ensure thorough mixing.
6. Cover the plate with a plate sealer and incubate at 25°C for 30 minutes in the dark.

7. Uncover the plate, discard the liquid. Immediately add 260 µl of 1X Wash Buffer to each well using a multi-channel pipette and wash. Wash 4 times (30 second soaking periods are recommended). *Complete removal of liquid between each wash is essential for good performance. 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.*
8. Add 100 µl of Detection Reagent B to each well and repeat steps 5 – 7.
9. Add 50 µl of TMB Substrate A and 50 µl of TMB Substrate B to each well and tap gently for 10 seconds to ensure thorough mixing.
10. Cover the plate with a plate sealer and incubate at 25°C for 15 minutes in the dark. *Incubation time is for reference only, do not exceed 30 minutes*. When an apparent gradient appears in the standard wells the reaction can be terminated.
11. Add 50 µl of Stop Solution to each well. It is important the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm within 5 minutes of the stop solution having been added.

C. Calculation of Results

This assay is competitive, therefore there is an inverse correlation between the Ivermectin concentration in the sample and the absorbance measured. Plot the Absorbance (%) on the y-axis, and the 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.

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

The absorbance (%) should be calculated as follows:

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

where:

A Average absorbance of standard/sample

A_0 Average absorbance of 0 ng/ml standard

D. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. The concentrated Wash Buffer may crystallize and separate. If this happens, please warm the tube, and mix gently to dissolve.
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 minutes.
5. Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. To avoid cross-contamination, do not reuse pipette tips and tubes.
8. Do not use expired components, or components from a different kit.
9. The substrate reagents should be used under sterile conditions, and light exposure should be minimized. Unused substrateshould be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.

Technical Support

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