Diclazuril (Dic) ELISA Kit

Catalog No.: abx365091

Size: 96 tests

Storage: Store at 2 - 8°C. Do not freeze.

Application: The quantitative detection of Diclazuril in tissue homogenates, eggs, muscle tissue and other biological samples.

Sensitivity: 0.3 ng/ml (ppb)

Detection Limits: Muscle, Eggs – 15 ppb

Sample Recovery Rate: 90% ± 30%

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

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Kit Components

1. 96-well microplate
2. Standards (1 ml each): 0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb
3. HRP Conjugate Reagent: 7 ml
4. Antibody Working Solution: 10 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 25 ml
9. Concentrated Sample Solution (20X): 25 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. Centrifuge
7. 50 ml centrifuge tubes
8. Absorbent filter papers
9. Nitrogen evaporator or water bath
10. Homogenizer
11. Vortex mixer
12. Deionized water

Reagents Required But Not Provided

1. Acetonitrile (CH_3CN)
2. Sodium chloride (NaCl)
3. N-hexane (C_6H_{14})
4. Ethanoic Acid (CH_3COOH)

Protocol

Bring all reagents and samples to room temperature before use.

Experimental apparatus should be clean and disposable pipette tips should be used to avoid cross-contamination during the experiment.

A. Preparation of Samples and Reagents

1. Reagents

- **Solution 1 – Ethanoic Acid Solution**

  Add 1 ml of Ethanoic Acid to 99 ml of deionized water. Mix thoroughly.

- **Solution 2 – Wash Buffer**

  Dilute the 20X Concentrated Wash Buffer with deionized water. Mix thoroughly.

- **Solution 3 – Sample Solution**

  Dilute the 20X Concentrated Sample Solution with deionized water. Mix thoroughly.

2. Sample Pretreatment

- **Eggs:** Homogenize the Egg sample. Weigh 1 g of homogenate into a 50 ml centrifuge tube, add 1 g NaCl, 1.5 ml of Ethanoic Acid Solution (Solution 1), 2 ml of N-hexane, and 4.5 ml of Acetonitrile, then vortex fully for 1 min. Centrifuge at 4000 g for 5 min. Discard the upper N-hexane layer liquid. Take 0.5 ml of the acetonitrile layer liquid and transfer to another centrifuge tube. Dry at 50 - 60°C using a nitrogen evaporator or a water bath. Dissolve the residue with 1 ml of Sample Solution (Solution 3), and mix fully for 30 seconds. Take 20 µl for analysis.

  Note: Sample dilution factor: 12; Detection limit: 15 ppb

- **Muscle:** Remove as much fat as possible from the sample, then homogenize and mix fully. Weigh 1 g of homogenate into a 50 ml centrifuge tube, add 0.1 ml of Ethanoic Acid Solution (Solution 1), 3 ml of N-hexane, and 6 ml of Acetonitrile, then vortex for 1 min. Centrifuge at 4000 g for 5 min. Discard the upper N-hexane layer liquid. Take 0.5 ml of the supernatant and transfer to a clean centrifuge tube. Dry at 50 - 60°C using a nitrogen evaporator or a water bath. Dissolve the residue with 1 ml of Sample Solution (Solution 3), and mix fully for 30 seconds. Take 20 µl for analysis.

  Note: Sample dilution factor: 12; 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.

B. Assay Procedure

Bring all reagents to room temperature prior to use. All reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. Number the sample and ordered standard wells, and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 20 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl HRP Conjugate Reagent to each well,
4. Add 80 µl Antibody Working Solution to each well.
5. Cover the plate with a plate sealer, shake gently for 5 seconds to mix, and incubate at 25°C for 30 min in the dark.
6. Carefully remove the plate sealer, and remove the liquid in each well. Immediately add 260 µl Diluted Wash Buffer (Solution 2) and wash.
7. Repeat this wash procedure 4 times, with 30 second intervals. Invert the plate and pat onto absorbent towels. Prick any bubbles remaining in the wells with a clean pipette.
8. Add 50 µl of Substrate Reagent A to each well.
9. Add 50 µl of Substrate Reagent B to each well. Shake gently for 5 seconds to ensure thorough mixing, and incubate at 25°C for 15 minutes in the dark. (The reaction time can be extended depending on the actual colour change.)
10. Add 50 µl of Stop Solution to each well, and shake gently to ensure thorough mixing.
11. Within 5 minutes of introducing the Stop Solution, determine the optical density of each well with a microplate reader at 450 nm.

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C. Analysis of Results

1. Calculating Sample Concentration from Standard Curve

This assay is competitive, therefore there is an inverse correlation between Diclazuril concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration on the y-axis, and absorbance measured on the x-axis (a semi-logarithmic plot). 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.

D. Precautions

Bring all reagents to room temperature prior to use.

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

D. Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit: https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

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