Carbendazim ELISA Kit

Catalog Number: EL2049-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

Carbendazim is a fungicide used for the treatment and control of fungal diseases. Carbendazim continues to be used, frequently in combination with other fungicides. The greatest use of Carbendazim occurs in Europe and Asia. It is among the twelve pesticides most frequently found in European Union (EU) monitoring programs. Carbendazim is a potential endocrine disruptor and animal studies have shown in utero exposure to cause severe physical deformities including the lack of formation of eyes and the development of hydrocephalus, or water on the brain. Studies have also shown reproductive effects including impaired testicular development and functioning and infertility. The European Commission has placed Carbendazim on a priority list

of chemicals affecting the function of hormones. Carbendazim is also highly toxic to aquatic life. The current EU MRLs for Carbendazim on fresh produce vary according to item but are in the range of 0.1-0.7 mg/kg. In the United States, Carbendazim is permitted for use only in paints and adhesives, in textiles, and for ornamental trees. It is not approved for use on foods; however, Carbendazim has been found in foods in the US, including baby food in 2000 and imported orange juice in 2012. The monitoring of water sources and food products, including fresh produce and juices, is necessary to ascertain that Carbendazim is not present at levels which present a danger to human health.

This kit is based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 1.5 hours in one operation, it can considerably minimize operation error and work intensity.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Carbendazim residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the carbendazim reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, carbendazim residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of carbendazim residue in honey, milk, juice, and rice.

4. Cross Reactions

Carbendazim 100%

Benzaldehyde ..... <0.1%

5. Equipment and Reagents Needed (not provided)

5.1 Equipment

- ELISA reader (450nm/630nm)
- Rotary evaporator or nitrogen drying instruments
- Vortex mixer
- Timer
- Wash bottle
- Analytical balance
- Polystyrene centrifuge tube: 50ml, 2ml
- Micropipettes: 20μl-200μl, 100μl-1000μl
- 300μl-multipipette

5.2 Reagents

- Deionized water

6. Components Provided in This Kit

- Microtiter plate with 96 wells coated with antigen
- Standard solutions (5 bottles×0.8ml/tube)
    - 0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb
- Spiking standard solution (0.8ml/tube) 1ppm
- Carbendazim Antibody#1: 11mL
- 100X HRP-Conjugated Antibody#2: 0.25mL
- Antibody#2 Diluent: 20mL
- 20X Wash solution: 28mL
- TMB Substrate solution: 12mL
- Stop solution: 14mL

7. Reagents Preparation

- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Antibody#2: combine one volume of the 100X HRP-Conjugated Antibody#2 with 99 volumes of Antibody#2 Diluent. Vortex for 10 seconds to mix.
  - ☛ Prepare this solution fresh before each test.

8. Notice and Precautions Before Operation

- Please use one tip in the process of experiment and change the tips when absorbing different reagent.
- The stop solution is 1 N hydrochloric acid, which is corrosive and an irritant. **Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.**
- If running more than two strips at once, the use of a multichannel pipette is required.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8ºC for 24h in dark.

9. Assay Process

9.1 Instructions Prior to Beginning Assay

- Ensure that all reagents and microwells are at room temperature (20-25℃). Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
- Return all the rest reagents to 2-8℃ immediately after their use.
- Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
- Avoid direct sunlight during the incubation; use the plate cover provided in the kit to cover the plate.

9.2 Steps in the Assay Process

- Take all reagents out at room temperature (20-25℃) for more than 30min. Shake gently before use.
- Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.
- The diluted wash solution should be brought to room temperature before use.
- Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
- Dispense 50μL of the Carbendazim standards, positive control, or sample into each well.
- Dispense 100μL of the Antibody#1 into appropriate test wells.
- Shake the plate gently for 30 seconds using a back-and-forth motion.
- Cover the plate. Incubate for 30 minutes at room temperature.
- Decant the contents of the wells into an appropriate waste container.
- Rinse the microwells with 250μL of the 1X wash solution for 3 times.
- Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
- Add 150μL of freshly prepared 1X HRP-conjugated Antibody#2 to each well.
- Shake the plate gently for 30 seconds using a back-and-forth motion.
- Cover the plate. Incubate for 30 minutes at room temperature.
- Decant the contents of the wells into an appropriate waste container.
- Rinse the microwells with 250μL of the 1X wash solution for 3 times.
- Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
- Add 100μL TMB substrate solution to each well, mix gently by shaking the plate manually and incubate for 5-15min at 25℃ with cover.
- Add 100μL the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5min after addition of stop solution).

NOTE: It is also possible to measure by eye without stop solution if there is no ELISA reader.

10. Results

10.1 Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

$$\text{Absorbance (\%)} = B / B_0 * 100$$

B = the mean absorbance value of each standards or each sample
B_0 = absorbance value of zero standard

10.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilog-arithmic of the concentration of the standards (ppb) as x-axis.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

11 Sensitivity, Accuracy and Precision

11.1 Test Sensitivity:

- Overall Sensitivity..... 0.1ppb

11.2 Detection limit:

- Milk/Juice/water 0.5ppb
- Honey 1ppb
- Rice/produce/processed food 1ppb

11.3 Precision:

- C.V. of the ELISA kit ..... less than 10%

12. General Instructions

12.1 Temperature of Reagents and Samples

- The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃). The antibody solution should be stored at 4℃, which will be used immediately after taking out. If the antibody solution is return to room temperature before assay, the OD values will be higher, and the result of the assay will not be right.

12.2 Microwells

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shaking of Reagents

- Shake each reagent gently before use.

12.4. Skin Protection

- Keep your skin away from the stop solution for it is the 1 N HCl solution.

12.5 Out of Date Kits

- Don't use kits that are out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 General Comments

- Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Special Issues Concerning Solutions and Reagents

- Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 Special Issues Concerning Color

- The coloration reaction needs 30min after the addition of solution A and solution B, but you can prolong the incubation time ranges to 35min or more if the color is too light to be determined, never exceed 40min, on the contrary, shorten the incubation time properly.

12.9 Incubation Temperatures

- Incubation temperature should be at room temperature (20-28℃). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

13. Storage

- Storage condition: 2-8℃
- Storage period: 12 months

14. About

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

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