



User's Manual

Pentachlorophenol ELISA Kit

REF

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

Pentachlorophenol belongs to the class of organochlorine pesticides and can be used as an insecticide, antibacterial agent, fungicide, algicide, and preservative. Pentachlorophenol continues to be used, frequently in combination with other pesticides and can easily accumulate in animals and can enter the human body through the food chain. Pentachlorophenol causes harmful effects on the liver, kidneys, blood, lungs, nervous system, immune system, gastrointestinal tract and is a probable human carcinogen.

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.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Pentachlorophenol 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 Pentachlorophenol residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Pentachlorophenol residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions (5 bottles×0.8ml/tube) 0ppb, 0.2ppb, 0.6ppb, 1.8ppb, 5.4ppb, 8.1ppb
3. Spiking standard solution (0.8ml/tube) 100ppb
4. Pentachlorophenol Antibody#1: 11mL
5. 100× HRP-Conjugated Antibody#2: 0.25mL
6. Antibody#2 Diluent: 20mL
7. 20× Wash solution: 28mL
8. TMB Substrate solution: 12mL
6. Stop solution: 14mL

Materials Required But Not Supplied

1. Equipment

1. ELISA reader (450nm/630nm)
2. Rotary evaporator or nitrogen drying instruments
3. Vortex mixer
4. Timer
5. Wash bottle
6. Analytical balance
7. Polystyrene centrifuge tube: 50ml, 2ml
8. Micropipettes: 20µl-200µl, 100µl-1000µl
9. 300µl-multipipette

2. Reagents

1. Deionized water

Storage

Storage condition: 2-8°C

Storage period: 12 months

Reagent Preparation

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

Assay Procedure

Instructions Prior to Beginning Assay

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

Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30min. Shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Dispense 50µL of the Pentachlorophenol standards, positive control, or sample into each well.
6. Dispense 100µL of the Antibody#1 into appropriate test wells.

7. Shake the plate gently for 30 seconds using a back-and-forth motion.
8. Cover the plate. Incubate for 30 minutes at room temperature.
9. Decant the contents of the wells into an appropriate waste container.
10. Rinse the microwells with 250µL of the 1× wash solution for 3 times.
11. Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
12. Add 150µL of freshly prepared 1× HRP-conjugated Antibody#2 to each well.
13. Shake the plate gently for 30 seconds using a back-and-forth motion.
14. Cover the plate. Incubate for 30 minutes at room temperature.
15. Decant the contents of the wells into an appropriate waste container.
16. Rinse the microwells with 250µL of the 1× wash solution for 3 times.
17. Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
18. Add 100µL TMB substrate solution to each well, mix gently by shaking the plate manually and incubate for 5-15min at 25°C with cover.
19. 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.

General Notes

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°C). The antibody solution should be stored at 4°C, 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.
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.
3. Shaking of Reagents: Shake each reagent gently before use.
4. Skin Protection: Keep your skin away from the stop solution for it is the in HCl solution.
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.
6. General Comments: Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
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 ($A_{450nm} < 0.5$).
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.
9. Incubation Temperatures: Incubation temperature should be at room temperature (20-28°C). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

Calculation

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 standard or each sample

B₀ = absorbance value of zero standard

2. Drawing a Standard Curve

To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic 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.

Precision

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

Detection Limit

Milk/Juice/water: 1ppb

Honey: 1ppb

Rice/produce/processed food: 1ppb

Sensitivity

Overall Sensitivity: 0.2ppb

Specificity

Pentachlorophenol: 100%

Precautions

1. Please use one tip in the process of experiment and change the tips when absorbing different reagent.
2. The stop solution is in 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.
3. If running more than two strips at once, the use of a multichannel pipette is required.
4. Make sure that all experimental instruments are clean.
5. Treated samples can be stored at 2-8°C for 24h in dark.