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User's Manual

Carbaryl ELISA Kit

REF **DEIANJ21**

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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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Carbaryl ELISA is an immunoassay for the detection of carbaryl in contaminated samples.

General Description

Carbaryl (1-naphthyl methylcarbamate) is a chemical in the carbamate family used chiefly as an insecticide. It is a cholinesterase inhibitor and is toxic to humans. It is classified as a likely human carcinogen by the United States Environmental Protection Agency (EPA.). The oral LD50 is 250 to 850 mg/kg for rats and 100 to 650 mg/kg for mice.[citation needed] A recent study reports that carbaryl is a structural mimic of the neurohormone melatonin and directly binds to MT2 melatonin receptor. This could significantly impact circadian rhythms and increase risk for diabetes and metabolic disorders.

Principles of Testing

The test is a direct competitive ELISA based on the recognition of Carbaryl by specific antibodies. Carbaryl, when present in a sample, and a Carbaryl-enzyme conjugate compete for the binding sites of anti-Carbaryl antibodies in solution. The Carbaryl antibodies are then bound by a second antibody immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Carbaryl present in the sample. The color reaction is stopped after a specified time and the color is evaluated using a microplate ELISA photometer. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided

1. Microtiter plate coated with a second antibody.
2. Carbaryl Standards.
3. Carbaryl-HRP Conjugate.
4. Conjugate Diluent.
5. Anti-Carbaryl Antibody Solution.
6. Sample Diluent.
7. Wash Solution.
8. Color (Substrate) Solution (TMB).
9. Stop Solution.

Materials Required But Not Supplied

1. Micro-pipettes with disposable plastic tips (20-200 µL)
2. Multi-channel pipette or stepper pipette (50-250 µL) with disposable plastic tips

3. Deionized or distilled water
4. Graduated cylinder
5. Container with 500 mL capacity (for 1X diluted Wash Solution)
6. Tape or Parafilm
7. Timer
8. Paper towels or equivalent absorbent material
9. Microtiter plate shaker (optional)
10. Microtiter plate washer (optional)
11. Microtiter plate reader (wave length 450 nm)

Storage

Store the kit at 2 - 8°C.

Assay Procedure

1. Add 50 µL of the standard solutions and samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of reconstituted enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
4. Incubate the strips for 60 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips five times using the 1× washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of substrate (color) solution to the wells using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 50 µL of stop solution to the wells using a multi-channel pipette or a stepping pipette in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.