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User's Manual

Carbendazim ELISA Kit

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Σ **96T**

RUO

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Carbendazim ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of Carbendazim in honey, milk, juice and rice, meat, Mango.

The unique features of the kit are:

1. Rapid (10 minutes), and organic reagent-free extraction method with high recovery (75 - 105%).
2. High sensitivity (0.5 ng/g or ppb) and low detection limit (1 ng/g or ppb for honey).
3. High reproducibility.
4. A quick ELISA assay (less than 2 hours regardless of number of samples).

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

1. Carbendazim Plate, 1 x 96-well Plate (8 wells x 12 strips)
2. Carbendazim Standards:
   Negative control (white cap tube)
   0.5ng/mL (yellow cap tube), 1.0 mL
   1.5ng/mL (orange cap tube), 1.0 mL
   3ng/mL (pink cap tube), 1.0 mL
   6ng/mL (purple cap tube), 1.0 mL
   12ng/mL (blue cap tube), 1.0 mL
   100ng/mL (spiking, red cap tube), 1.0 mL
3. Carbendazim Antibody #1, 6ml
4. 100X HRP-Conjugated Antibody #2, 250 uL
5. Antibody #2 Diluent, 20 mL
6. 20X Wash Solution **, 30 mL
7. Stop Buffer **, 14 mL
8. TMB Substrate **, 10 mL
9. 10X Sample Extraction Buffer F, 25 mL

10. Concentrate of CBD Extraction Buffer, 30g
11. CBD Clean Up Reagent, 25g
12. 10X Mango Extraction Buffer(Optional), 20 mL
13. 100X Mango Balance Buffer (Optional), 1mL
14. 20X CBD Honey R. Bf.(Optional), 6ml

* If you are not planning to use the kit for over 3 months, store Carbendazim Antibody #1 and 100X HRP-Conjugated Antibody #2 at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Vortex mixer (e.g. Gneie Vortex mixer from VWR)
3. 10, 20, 100 and 1000 uL pipettes
4. Multi-channel pipette: 50-300 uL (Optional)

Storage

Carbendazim ELISA Kit has the Carbendazim for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. Store the kit at 2-8°C *. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 - 25°C / 68 - 77°F) or in a refrigerator before use.

1. **Preparation of 1X Sample Extraction Buffer**

   Mix 1 volume of 10X Sample Extraction Buffer with 9 volumes of distilled water.

2. **Preparation of 1X CBD Extraction Buffer**

   Take all of the powder from the Concentrate of CBD Extraction Buffer bag to a 125-mL bottle, add 90 mL of distilled water, vortex 2 minutes manually, leave the solution at room temperature for 20 minutes. It is fine if small amount of salt residue is observed at the bottom of the bottle.

3. **Preparation of 1X CBD Honey Resuspension Buffer**

   Mix 1 volume of 20X CBD Honey Resuspension Buffer with 19 volumes of distilled water.

4. **Preparation of 1% Acetic Acid / Acetonitrile**

   Mix 1 mL of 100% acetic acid with 99 mL of acetonitrile.

5. **Preparation of 1X Mango Extraction Buffer**

   Mix 1 volume of 10X Mango Extraction Buffer with 9 volumes of distilled water.

6. **Preparation of 100X Mango Balance Buffer**

Mix 1 volume of 100X Mango Balance Buffer with 99 volumes of distilled water.

Honey

1. Dissolve 1 g of honey with 1 mL of 1X CBD Extraction Buffer in a 15 mL centrifugal vial.
2. Add 4 mL of 1% Acetic Acid / Acetonitrile solution. Vortex for 3 minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
3. Centrifuge the sample for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77 °F).
4. Transfer 1.6 mL of the acetonitrile supernatant to a new tube containing 300 mg (280-320 mg) of CBD Clean Up Reagent. Vortex for 30 seconds at maximum speed and leave at room temperature for at least 5 minutes.
5. Centrifuge the sample for 5 minutes at 4,000 x g at room temperature (20 - 25 °C / 68 - 77 °F).
6. Transfer 600 uL of the supernatant to another tube (corresponding to 0.15 g of the original sample) and use a rotary evaporator to dry the sample at 60 - 70°C under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas over it in a 60 - 70 °C water bath.
7. Dissolve the dried residue in 1.5 mL of 1X CBD Honey Resuspension Buffer and vortex vigorously for 1 minute. Put the samples in a 60°C incubator or a 60°C heat block for 10 minutes. Vortex samples again for 1 minute to ensure all the residue is dissolved.
8. Use 50 uL of the sample per well for the assay.

   Note: Dilution factor: 10.

Juice/Milk

1. Add 0.8 mL of 1X CBD Extraction Buffer, 0.2 mL Mango Balance Buffer and 4 mL of Acetonitrile to 1 mL of juice/milk sample, vortex for 1 minute at maximum speed.
2. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F)
3. Transfer 1.6 mL of the Acetonitrile supernatant to another tube, add about 300 mg (280-320 mg) CBD Clean Up Reagent, vortex for 30 seconds at maximum speed, leave at room temperature at least 5 minutes.
4. Transfer 1.0 mL of the supernatant to another tube (corresponding to 0.25 mL of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 0.5 mL of 1X Sample Extraction Buffer by vortexing at maximum speed for 1 minute.
6. Use 50 uL of the sample per well for the assay

   Note: Dilution factor: 2.

Mango Puree

1. To 1 mL of liquid Mango Puree, add 10 uL of Mango Balance Buffer. Vortex the sample for 20- 30 seconds.
2. Add 1.990 mL of 1X Mango Extraction Buffer to the sample.
3. Vortex the sample for 3 - 5 minutes.
4. Centrifuge the sample for 10 minutes at 4,000 x g at room temperature.
5. Use 50 uL of supernatant per well in the assay.

   Note: Dilution factor: 3.

Meat

1. Add 1 mL of 1X CBD Extraction Buffer and 4 mL of Acetonitrile to 1 g of meat sample, vortex for 3 minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
2. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F).
3. Transfer 1.6 mL of the Acetonitrile supernatant to another tube, add about 300 mg (280-320 mg) CBD Clean Up Reagent, vortex for 30 seconds at maximum speed, leave at room temperature at least 5 minutes.
4. Transfer 1.2 mL of the supernatant to another tube (corresponding to 0.3 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 0.3 mL of 1X Sample Extraction Buffer F by vortexing at maximum speed for 1 minute.
6. Use 50 uL of the sample per well for the assay.

   Note: Dilution factor: 1.

Rice

1. Add 2 mL of 1X CBD Extraction Buffer and 4 mL of Acetonitrile to 1 g of rice sample, vortex for 3 minutes at maximum speed.
2. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F)
3. Transfer 1.6 mL of the Acetonitrile supernatant to another tube, add about 300 mg (280-320 mg) CBD Clean Up Reagent, vortex for 30 seconds at maximum speed, leave at room temperature at least 5 minutes.
4. Transfer 1.0 mL of the supernatant to another tube (corresponding to 0.25 mL of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 0.5 mL of 1X Sample Extraction Buffer by vortexing at maximum speed for 1 minute.
6. Use 50 uL of the sample per well for the assay

   Note: Dilution factor: 2.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 - 2 hours at 20 - 25°C / 68 - 77°F); Make sure you read "Precautions" section. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. **Preparation of 1X HRP-Conjugated Antibody #2**

   Mix 1 volume of 100X Antibody #2 with 99 volumes of Antibody #2 Diluent.

2. **Preparation of 1X Wash Solution**

   Mix 1 volume of 20X Wash Buffer concentrate with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Carbendazim Antibody #1	50μL	1.2mL
1X HRP-Conjugated Antibody #2	150μL	3.6 mL
1X Wash Solution	2.0 mL	48mL
Stop Buffer	100 μL	2.4 mL
TMB Substrate	100 μL	2.4 mL

1. Add 50uLof each Cyproheptadine Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50uL of each sample in duplicate into different sample wells.
3. Add 50 uL Antibody #1 to each well and mmix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 - 25°C / 68 - 77°F).
5. Wash the plate 3 times with 250 uL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 150uL of 1X Antibody #2 to each well, incubate the plate for 30 minutes at room temperature (20 - 25°C / 68 - 77°F).
7. Wash the plate 3 times with 250 uL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
8. Add 100uL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating. (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).
9. After incubation for 10-15 minutes in room temperature, add 100 uL of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

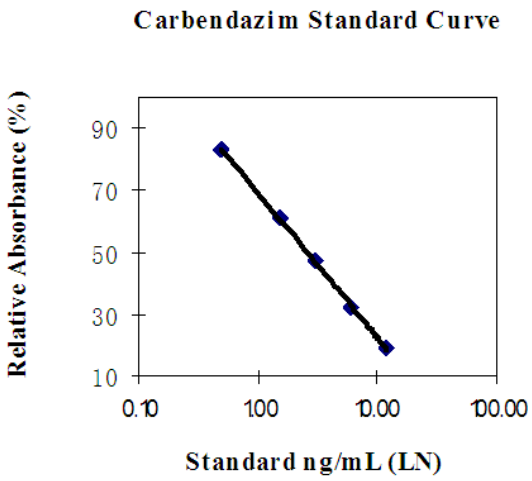
Calculation

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 / absorbance zero standard

Typical Standard Curve

The following figure is a typical Carbendazim standard curve



Sensitivity

Honey: 5ppb

Juice: 2ppb

Rice feed: 2ppb

Mango: 3ppb

meat: 1ppb

Specificity

Carbendazim: 100%

Mebendazole: 100%

Fenbendazole: 80%

4' -hydroxyfenbendazole: 48%

Benzaldehyde: <0.1%

Cambendazole: <0.1%

Precautions

1. The standards contain Carbendazim. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates.
4. Try to maintain a laboratory temperature of 20-25°C (68-77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.

6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 - 25°C / 68 - 77°F) while in the packaging.