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THC HAIR KIT

ELISA KIT INSTRUCTIONS PRODUCT #140619, 140615 & 140613
FORENSIC USE ONLY

INTENDED USE: For the determination of trace quantities of THC and/or other metabolites in human hair samples.

DESCRIPTION

Neogen Corporation's THC Hair ELISA (Enzyme-Linked Immunosorbent Assay) test kit is a qualitative one-step kit designed for use as a screening device for the detection of drugs and/or their metabolites. The kit was designed for screening purposes and is intended for forensic use only. It is recommended that all suspect samples be confirmed by a quantitative method such as gas chromatography/mass spectrometry (GC/MS).

ASSAY PRINCIPLE

The Neogen forensic drug detection ELISA is a solid phase immunoassay designed to detect drugs of abuse for forensic application. The test is performed in microwells coated with a high affinity capture antibody. A control or sample is added to the wells followed by an enzyme conjugate. During the following incubation period, the enzyme conjugate competes with the drug in the sample for binding sites on the antibody coated well. After a wash step to remove any unbound material, substrate is added for the color development process. Acid stop solution is added to discontinue the enzyme-substrate reaction. The color intensity is inversely proportional to the amount of drug present in the sample. Therefore, those samples which contain the drug will inhibit binding of the enzyme conjugate to the capture antibody resulting in less color than the negative control. Negative and positive controls should be run along with the samples. Results should be obtained by reading the absorbance of the wells with a microplate reader.

STORAGE AND STABILITY

This kit can be used until the expiration date on the label and Certificate of Analysis when stored refrigerated between 2 - 8°C. Always check each kit for specific expiration dates and storage requirements.

MATERIALS PROVIDED – SINGLE KIT (96 WELL)

1. **Wash Buffer Concentrate (10X):** 2 X 20 mL. Phosphate buffered saline solution with a surfactant. Dilute 10 fold with deionized or ultrapure water before use. Diluted wash buffer is used to wash all unbound conjugate and samples from the plate after the conjugate incubation.
2. **K-Blue® Substrate:** 20 mL (ready-to-use). Stabilized 3,3',5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle. It is used to develop the color in the wells after washing. Light-sensitive.
3. **Drug-Enzyme Conjugate:** 200 µL. Drug-horseradish peroxidase conjugate. Dilute 100X before use.
4. **Drug-Enzyme Diluent:** 15 mL.
5. **Acid Stop Solution:** 18 mL of 1N H_2SO_4.
6. **Antibody-coated Plate:** 96 well plate coated with anti-drug antiserum. The plate is ready for use. Do not wash.

MATERIALS PROVIDED – BULK KIT (480 WELL)

1. **Wash Buffer Concentrate (10X):** 2 X 100 mL. Phosphate buffered saline solution with a surfactant. Dilute 10 fold with deionized or ultrapure water before use. Diluted wash buffer is used to wash all unbound conjugate and samples from the plate after the conjugate incubation.
2. **K-Blue Substrate:** 100 mL (ready-to-use). Stabilized 3,3',5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle. It is used to develop the color in the wells after washing. Light-sensitive.
3. **Drug-Enzyme Conjugate:** 1 mL. Drug-horseradish peroxidase conjugate. Dilute 100X before use.
4. **Drug-Enzyme Diluent:** 85 mL.
5. **Acid Stop Solution:** 90 mL of 1N H_2SO_4.
6. **Antibody-coated Plate:** 5 X 96 well plate coated with anti-drug antiserum. The plate is ready for use. Do not wash.

MATERIALS PROVIDED – 50 PACK (4800 WELL)

1. **Wash Buffer Concentrate (10X):** 1 L. Phosphate buffered saline solution with a surfactant. Dilute 10 fold with deionized or ultrapure water before use. Diluted wash buffer is used to wash all unbound conjugate and samples from the plate after the conjugate incubation.
2. **K-Blue Substrate:** 500 mL (ready-to-use). Stabilized 3,3',5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle. It is used to develop the color in the wells after washing. Light-sensitive.
3. **Drug-Enzyme Conjugate:** 1 mL. Drug-horseradish peroxidase conjugate. Dilute 100X before use.
4. **Drug-Enzyme Diluent:** 850 mL.
5. **Acid Stop Solution:** 500 mL of 1N H_2SO_4.
6. **Antibody-coated Plate:** 50 X 96 well plate coated with anti-drug antiserum. The plate is ready for use. Do not wash.

MATERIALS NEEDED BUT NOT PROVIDED

1. Dilution Buffer (0.1% BSA/PBS). Dilution Buffer referenced in the Extraction Procedure can be ordered separately (Product no. 301777) or may be made by the laboratory.
2. Precision pipettes that range from 10 uL - 1000 uL and disposable tips.
3. Graduated cylinder to dilute and mix wash buffer.
4. Clean glassware (i.e. test tubes) to complete hair extraction procedure.
5. Microplate reader capable of measuring absorbance at 450 nm (650 nm, 630 nm, or 620 nm wavelength reference filter).
6. Borosilicate glass culture tubes or comparable vessels for use with controls, standards and hair extraction procedure. PLASTICS CANNOT BE USED.
7. Methanol.
8. Heating Block.
9. Deionized water.
10. Nitrogen (gas).

PRECAUTIONS AND NOTES

1. **DO NOT** use kits or components beyond expiration date.
2. **DO NOT** mix conjugates and plates from different kit lots.
3. **DO NOT** pipette reagents by mouth.
4. Pour K-Blue Substrate out of the bottle into a clean reservoir. To prevent contamination of the substrate, **DO NOT** pipette out of the bottle.
5. All specimens should be considered potentially infectious. Exercise proper handling precautions.
6. Keep plate covered except when adding reagents, washing or reading.
7. Kit components should be refrigerated at all times when not in use.
8. Use aseptic technique when opening and removing reagents from vials and bottles.
9. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
10. Do not substitute DI water for the wash step of this protocol. Use only Neogen's wash buffer.
11. Do not use Sodium Azide with samples, standards and/or calibrators.
12. Do not reuse wells, they are for one-time use only.

PROCEDURAL NOTES

1. Desiccant bag must remain in foil pouch with unused strips. Keep zipped bag sealed when not in use to maintain a dry environment.
2. Use clean pipette tips for the buffer, drug-enzyme conjugate, controls and samples.
3. Before pipetting a reagent, rinse the pipette tip three times with that reagent.
4. When pipetting into the wells, **DO NOT** allow the pipette tip to touch the inside of the well or any of the reagent already inside the well. This may result in cross contamination.
5. Controls and samples should be assayed in duplicate.
6. Before opening the drug-enzyme conjugate vial, tap the vial in an upright position to remove any liquid in the cap.
7. Before substrate addition, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.
8. Gently mix specimens and reagents before use. Avoid vigorous agitation.

SAMPLE TREATMENT

Prepare hair samples in accordance with the lab's internal method or with the method recommended below.

HAIR EXTRACTION PROCEDURE

1. Weigh out a 20 mg portion of each hair sample and place into a labeled 16 x 100-mm disposable borosilicate glass culture tubes or comparable vessels.
2. Wash Step for hair: Add 2 mL of Methanol to each tube and incubate for 10 minutes at room temperature, swirling occasionally.
3. Decant the solvent by pouring off the Methanol solution.
4. Add 2 mL of fresh Methanol to the tube and incubate for 2 hours at 70 – 75°C. Place a punctured test tube cap onto the borosilicate glass culture tubes or comparable vessels.
5. Cool the tube to room temperature and transfer the Methanol to a clean 12 x 75-mm disposable borosilicate glass culture tubes or comparable vessels.
6. Evaporate the Methanol with a stream of nitrogen in a heating block at 37°C.
7. Add 600 µL of Dilution Buffer (0.1% BSA/PBS) to the residue to reconstitute. Vortex.
8. Follow the Test Procedures.

Reference: Sweeney, Stacy A. *et al*. "Amphetamines in Hair by Enzyme-Linked Immunosorbent Assay". Journal of Analytical Toxicology. Vol. 22, October 1998.

ENZYME PREPARATION

1. Prior to use, perform a 100X dilution of the drug-enzyme conjugate using the provided drug-enzyme diluent. The drug-enzyme conjugate is most stable in its concentrated form. Dilute only the volume necessary for the amount of strips currently being used. For example:

# of plates	Volume of Conjugate	Volume of Drug-Enzyme Diluent
1	140 µL	13.86 mL
5	700 µL	69.3 mL
25	3.5 mL	346.5 mL

2. Gently mix the diluted drug-enzyme conjugate solution by inverting 10-15 times. **Do not vortex**. Store unused conjugate at 4°C.

TEST PROCEDURES

The following test procedures can be run manually or on an automated instrument. Please contact your Neogen representative for assistance with protocols for automated instruments.

1. Determine the number of wells and amounts of reagents that will be required for immediate testing. Remove any excess strips from the strip holder. Store them in the zipped bag that previously contained the full plate. (Neogen does not recommend breaking strips.) Dispense the necessary quantity of diluted enzyme. Return the unused strips and enzyme to the appropriate storage conditions.
2. All ELISA components, controls, and samples must be at room temperature, i.e., 20-23°C (68-74°F) prior to use. Mix reagents by gentle inversion of bottles.
3. Pipette 100 µL of a negative control (blank) into wells. Controls and samples should be added directly to the bottom of the wells.
4. Add 100 µL of positive controls (low, cutoff calibrator, and high) into wells.
5. Add 100 µL of each prepared sample into wells.
6. Allow the reaction to incubate at room temperature for 60 minutes. Neogen recommends gentle agitation during this time, preferably on a plate shaker.
7. Dump the solution from the wells. Wash the wells by dispensing 350 µL of diluted wash buffer to each well and then aspirating to remove the solution from the wells. Manual Wash: For manual wash procedures, repeat for a total of 5 washes. Finally, invert and firmly tamp the strip holder on absorbent paper to remove the last traces of liquid from the wells. Automated Wash: If an automated plate washer is used, wash the plate for a total of 5 washes with 350 µL of diluted wash buffer. It is important for the automated washer to conduct a final aspirate cycle to eliminate residual amounts of wash buffer. Residual amounts of buffer in the wells will affect assay performance. **Note**: DI water should never be used for the plate wash.
8. Immediately add 100 µL of the diluted enzyme (refer to Enzyme Preparation) to each well. Allow the reaction to incubate at room temperature for 30 minutes, while shaking.
9. Repeat Step 7 for a second wash step. Dump the solution from the wells. Wash the wells by dispensing 350 µL of diluted wash buffer to each well and then aspirating to remove the solution from the wells. Manual Wash: For manual wash procedures, repeat for a total of 5 washes. Finally, invert and firmly tamp the strip holder on absorbent paper to remove the last traces of liquid from the wells. Automated Wash: If an automated plate washer is used, wash the plate for a total of 5 washes with 350 µL of diluted wash buffer. It is important for the automated washer to conduct a final aspirate cycle to eliminate residual amounts of wash buffer. Residual amounts of buffer in the wells will affect assay performance. **Note**: DI water should never be used for the plate wash.

10. Proceed immediately to Substrate addition. Ensure that the outside bottoms of the wells are clean and dry. Dispense the necessary volume of Substrate from the storage bottle into an intermediate vessel such as a clean reagent trough or weigh boat. From the intermediate vessel, transfer 100 µL of the Substrate to each well.
11. Allow substrate reaction to proceed for 15 minutes. Neogen recommends gentle agitation during this time, preferably on a plate shaker.
12. Stop the reaction by adding 100 µL of Acid Stop Solution to each well containing Substrate (do not dump or aspirate Substrate).
13. Using a microplate reader, determine the absorbance of each well at 450 nm. The optimal wavelengths to select are 450 nm (absorbance) and 650 nm, 630 nm, or 620 nm (reference).

SENSITIVITY/SPECIFICITY

Compound	Compound Concentration (pg/mg)	(-) Δ^9-THC Equivalents (pg/mg)	% Cross-Reactivity
(-)-11-nor-9-Carboxy-Δ^8-THC	1.0	1.5	150%
(-)-11-nor-9-Carboxy-Δ^9-THC	1.0	1.5	150%
(±)-11-Hydroxy-Δ^9-THC	1.4	1.5	107%
(-)-Δ^9-THC	1.5	1.5	100%
(±)-11-nor-9-Carboxy-Δ^9-THC	1.8	1.5	83%
(-)-Δ^8-THC	2.1	1.5	71%
Cannabinol (CBN)	3.4	1.5	44%
Cannabidiol (CBD)	4,800	1.5	0.03%

Note: (-)-Δ^9-THC equivalents represent 50% B/B_0 assay displacement in BPS Buffer.

RESULTS INTERPRETATION

Each laboratory should determine the cutoff level for their individual application. When possible, cutoff calibrators and/or standards should be prepared in the same matrix being tested.

Positive Result: Samples with an absorbance less than or equal to the laboratory's designated cutoff calibrator should be considered positive. All positive samples should be confirmed by a quantitative method such as GC/MS.

Negative Result: Samples with an absorbance greater than the laboratory's designated cutoff calibrator should be considered negative.

Note: The kit was designed for screening purposes only. It is recommended that all suspect samples be confirmed by a quantitative method such as GC/MS or HPLC.

TECHNICAL SUPPORT

For technical assistance, please contact our Technical Services Department at (859) 254-1221 or email at techservice-toxicology@neogen.com. Representatives are available Monday – Friday from 8:00 am – 6:00 pm EST.

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