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SYNTHETIC CATHINONES (METHCATHINONE)

ELISA KIT INSTRUCTIONS PRODUCT #181819, 181815, & 181813
FORENSIC USE ONLY

INTENDED USE: For the determination of trace quantities of Synthetic Cathinones (Methcathinone) and/or other metabolites in human blood, serum and oral fluid.

DESCRIPTION

Neogen Corporation's Synthetic Cathinones (Methcathinone) ELISA (Enzyme-Linked ImmunoSorbent Assay) test kit is a qualitative 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 PRINCIPLES

Neogen Corporation's test kit operates on the basis of competition between the drug or its metabolite in the sample and the drug-enzyme conjugate for a limited number of antibody binding sites. First, the sample or control is added to the microplate. Next, the drug-enzyme conjugate is added and the mixture is incubated at room temperature. During this incubation, the drug in the sample or the drug-enzyme conjugate binds to antibody immobilized in the microplate wells. After incubation, the plate is washed to remove any unbound sample or drug-enzyme conjugate. The presence of bound drug-enzyme conjugate is recognized by the addition of K-Blue® Substrate (TMB). After a 30 minute substrate incubation, the reaction is halted with the addition of an acid stop. The test can be read visually or with a microplate reader equipped with a 450 nm filter. The extent of color development is inversely proportional to the amount of drug in the sample or control. In other words, the absence of the drug in the sample will result in a dark yellow color, whereas the presence of the drug will result in light yellow to no color development.

STORAGE AND STABILITY

This kit can be used until the expiration date on the label when stored refrigerated at 2-8°C. Store controls frozen if not used within 10 days. Note: Some kits require controls to be stored frozen immediately upon receipt. Reference kit label for details.

MATERIALS PROVIDED – SINGLE KIT (96 WELL)

1. **EIA Buffer:** 30 mL (ready-to-use). Phosphate buffered saline solution with bovine serum and a preservative. Provided for dilution of samples.
2. **Wash Buffer Concentrate (10X):** 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.
3. **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.
4. **Drug-Enzyme Conjugate:** 200 µL. Drug-horseradish peroxidase conjugate. Dilute 180X before use.
5. **Synthetic Cathinones (Methcathinone) Enzyme Conjugate Diluent:** 23 mL (ready-to-use). Provided for dilution of drug-enzyme conjugate. **Note:** Diluent is specific for the Synthetic Cathinones (Methcathinone) drug-enzyme conjugate.
6. **Antibody Coated Plate:** A 96-well Costar plate, in strips of 8 break-away wells, coated with anti-drug antiserum. The plate is ready for use as is. Do not wash.
7. **Acid Stop Solution:** 14 mL (ready-to-use). 1N H_2SO_4 used to stop the enzyme reaction.
8. **Qualitative QC Positive Control:** 750 µL provided (synthetic matrix). Do not dilute.
9. **Qualitative QC Negative Control:** 750 µL provided (synthetic matrix). Do not dilute.

MATERIALS PROVIDED – BULK KIT (480 WELL)

1. **EIA Buffer:** 200 mL (ready-to-use). Phosphate buffered saline solution with bovine serum and a preservative. Provided for dilution of samples.
2. **Wash Buffer Concentrate (10X):** 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.
3. **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.
4. **Drug-Enzyme Conjugate:** 1 mL. Drug-horseradish peroxidase conjugate. Dilute 180X before use.
5. **Synthetic Cathinones (Methcathinone) Enzyme Conjugate Diluent:** 5 X 23 mL (ready-to-use). Provided for dilution of drug-enzyme conjugate. **Note:** Diluent is specific for the Synthetic Cathinones (Methcathinone) drug-enzyme conjugate.
6. **Antibody Coated Plate:** 5 X 96 well Costar plates, in strips of 8 break-away wells, coated with anti-drug antiserum. The plates are ready for use as is. Do not wash.
7. **Acid Stop Solution:** 90 mL (ready-to-use). 1N H_2SO_4 used to stop the enzyme reaction.

MATERIALS PROVIDED – 50 PACK (4800 WELL)

1. **EIA Buffer:** 500 mL (ready-to-use). Phosphate buffered saline solution with bovine serum and a preservative. Provided for dilution of samples.
2. **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.
3. **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.
4. **Drug-Enzyme Conjugate:** 9 mL. Drug-horseradish peroxidase conjugate. Dilute 180X with EIA Buffer prior to use.
5. **Synthetic Cathinones (Methcathinone) Enzyme Conjugate Diluent:** 850 mL (ready-to-use). Provided for dilution of drug-enzyme conjugate. **Note:** Diluent is specific for the Synthetic Cathinones (Methcathinone) drug-enzyme conjugate.
6. **Antibody Coated Plate:** 50 x 96 well Costar plate, in strips of 8 break-away wells, coated with anti-drug antiserum. The plate is ready for use as is. Do not wash.
7. **Acid Stop Solution:** 500 mL (ready-to-use). 1N H_2SO_4 used to stop the enzyme reaction.

OPTIONAL TEST MATERIALS

1. **Qualitative QC Positive Control:** 5 X 750 µL (synthetic matrix). 1-5 vials available upon request. Do not dilute.
2. **Qualitative QC Negative Control:** 5 X 750 µL (synthetic matrix). 1-5 vials available upon request. Do not dilute.

MATERIALS NEEDED BUT NOT PROVIDED

1. Deionized water.
2. Precision pipettes that range from 10 µL - 1000 µL and disposable tips.
3. Graduated cylinder to dilute and mix wash buffer.
4. Plate cover or plastic film to cover plate during incubation.
5. Clean glassware (i.e. test tubes) to dilute samples.
6. Microplate reader with 450 nm filter.
7. Cutoff calibrator.

OPTIONAL MATERIALS

1. Microplate shaker
2. NeoSal Buffer, 1L Product No. 120177 and 500 mL Product No. 120176

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. Keep the controls frozen if storing longer than 10 days. Avoid repeated freeze-thaw cycles. Note: Some kits require controls to be stored frozen immediately upon receipt. Reference kit label for details.
9. Use aseptic technique when opening and removing reagents from vials and bottles.
10. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
11. **DO NOT** substitute DI water for the wash step of this protocol. Use only Neogen's wash buffer.
12. Sodium Azide concentrations at 0.01% or less should not interfere with the assay provided that recommended dilutions are followed.
13. **DO NOT** reuse wells, they are for one use only.

PROCEDURAL NOTES

1. Desiccant bag must remain in foil pouch with unused strips. Keep ziplock pouch 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 substrate addition, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.
7. Gently mix specimens and reagents before use. Avoid vigorous agitation.

SAMPLE TREATMENT

Recommended minimum sample dilutions are listed below. These dilutions may change based on your laboratory's determination. All sample dilutions should be made in Neogen's EIA Buffer.

a. **Whole blood:** A dilution of 1:20 (i.e. 1 part sample to 19 parts provided EIA buffer) is required for optimal assay performance.
b. **Serum:** A dilution of 1:5 (i.e. 1 part sample to 4 parts provided EIA buffer) or 1:20 (i.e. 1 part sample to 19 parts provided EIA buffer) is required for optimal assay performance.
c. **Oral Fluid:** A dilution of 1:4 (i.e. 1 part sample to 3 parts NeoSal Buffer*) is required for optimal assay performance.
d. **Urine:** Due to interferences by high concentrations of ephedrine and pseudoephedrine commonly encountered in human urine samples, Neogen's Synthetic Cathinones (Methcathinone) ELISA is recommended for human whole blood, serum and oral fluid matrices. If you plan on using this assay for human urine samples, please contact your Neogen Representative for assistance.
e. **Other Forensic sample types:** Please contact your Neogen Representative for assistance.

*NeoSal Buffer is used with the NeoSal Oral Fluid Collection System and is sold separately.

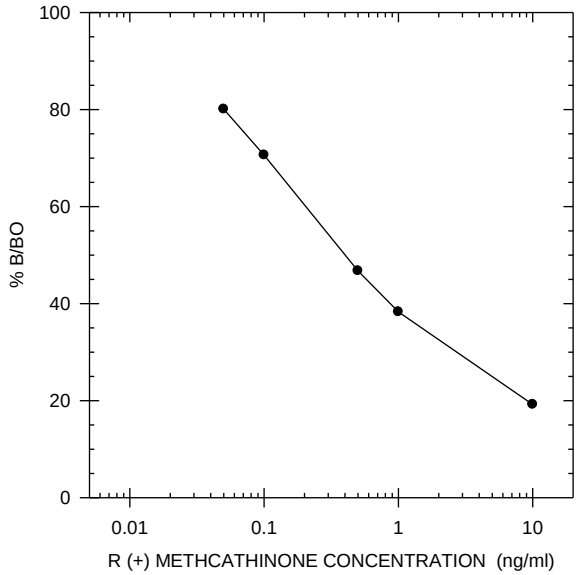
TEST PROCEDURES

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 to be used.
2. Prepare the enzyme conjugate solution by diluting the enzyme conjugate stock 1 to 180 in the Synthetic Cathinone (Methcathinone) Conjugate Diluent provided. Mix the solution by inversion. Do not vortex. Example: for two eight well strips, add 25 µL of the enzyme conjugate stock to 4475 µL of Synthetic Cathinones (Methcathinone) Enzyme Conjugate Diluent. Store unused conjugate at 2-8°C.
3. Add 20 µL of sample, Neogen controls or laboratory calibrators to the appropriate wells in duplicate. DO NOT dilute Neogen's positive or negative controls
4. Add 100 µL of diluted drug-enzyme conjugate to each well. Use 8-channel pipetter or 12-channel pipetter for rapid addition.
5. For manual runs, mix by gently shaking plate. A microplate shaker may be used.
6. Cover plate with plastic film or plate cover and incubate at room temperature for 45 minutes.
7. During the conjugate incubation, dilute concentrated wash buffer 10 fold with deionized water (i.e. 20 mL of concentrated wash buffer plus 180 mL of deionized water). Mix thoroughly. Diluted wash buffer is stable for 5 days at room temperature or 7 days at 2-8°C.
8. Once the incubation is complete, dump or aspirate the liquid from the wells. Tap the plate on a clean lint-free towel to remove any remaining liquid in the wells.

9. Wash each well with 300 µL of diluted wash buffer. Manual Wash: For manual wash procedures repeat for a total of 3 washings, invert and tap dry the plate following each step. After completing the last wash step wipe the bottom of the wells with a lint-free towel to remove any liquid on the outside of the wells. Automated Wash: If an automated plate washer is used wash the plate for a total of 5 washings with 300 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. Add 100 µL of the K-Blue Substrate to each well. For manual runs, use a multi-channel pipetter for best results.
11. Incubate at room temperature for 30 minutes.
12. Add 100 µL of the Acid Stop (1N H_2SO_4) to each well to stop enzyme reaction. Mix gently before measuring the absorbance. For automated systems a 10 second shake is sufficient. Measure the absorbance at a wavelength of 450 nm. Wells should be read within 2 hours of stopping the reaction.

STANDARD CURVE IN EIA BUFFER



SENSITIVITY

Compound	***I-50 in EIA Buffer***	***I-50 in Whole Blood (1:20 Dilution: 1 part sample to 19 parts EIA buffer)***	***I-50 in Serum (1:5 Dilution: 1 part sample to 4 parts EIA buffer)***	***I-50 in Oral Fluid (1:4 Dilution: 1 part sample to 3 parts NeoSal Buffer*)***
R(+) Methcathinone	0.30 ng/ml	7.5 ng/mL	1.4 ng/mL	1.3 ng/mL

*NeoSal Buffer is used with the NeoSal Oral Fluid Collection System and is sold separately.

The term I-50 is used to define the sensitivity of the test. This number is derived from a standard curve generated with the drug in EIA Buffer. The drug concentration that shows 50% less color activity than the zero standard is considered to be the I-50.

SPECIFICITY

Compound	*Compound Concentration (ng/mL)*	*R(+) Methcathinone Equivalents (ng/mL)*	*% Cross Reactivity*
R(+) Methcathinone	**0.30**	**0.30**	**100**
Mephedrone	0.18	0.30	167
Methedrone	0.2	0.30	150
Methylone	0.25	0.30	120
Buphedrone	0.29	0.30	103
4-Fluoromethcathinone (Flephedrone)	0.32	0.30	94
3-Fluoromethcathinone	0.4	0.30	75
(±) Methcathinone	0.41	0.30	73
Ethylone (bk-MDEA)	0.7	0.30	43
Ethcathinone	0.75	0.30	40
4-Methylethcathinone	0.85	0.30	35
Pentylone	15	0.30	2
Pentedrone	21	0.30	1
R(+) Cathinone	70	0.30	0.43
S(+) Methamphetamine	340	0.30	0.088
(±) MBDB (Butylone)	550	0.30	0.055
(-)-(S)-Cathinone	600	0.30	0.050
1R,2S(-) Ephedrine	700	0.30	0.043
1S,2R(+) Ephedrine	2,600	0.30	0.012
MDMA	3,083	0.30	0.010
Diethylpropion	4,700	0.30	0.006
(±) N-Ethylcathinone ephedrine	5,000	0.30	0.006
R,R(-) Pseudoephedrine	6,000	0.30	0.005
MDPV	>10,000	0.30	< 0.003
PVP	>10,000	0.30	< 0.003
(±) MDA	>10,000	0.30	< 0.003
R(-) Amphetamine	>10,000	0.30	< 0.003
(+) Norpseudoephedrine	>10,000	0.30	< 0.003
(+)-N,N-Diethylnorephedrine	>10,000	0.30	< 0.003
(±) Phenylpropanolamine	>10,000	0.30	< 0.003
Naphyrone	>10,000	0.30	< 0.003
S,S(+)-Pseudoephedrine	>10,000	0.30	< 0.003

Note: R(+) Methcathinone equivalents represent 50% B/B_0 assay displacement in EIA Buffer.

The compounds having cross-reactivity below 0.01% did not show any significant reaction up to 10µg/mL.

ALL THE FOLLOWING HAVE A CROSS-REACTIVITY <0.01%.

Acepromazine; Acetaminophen; Acetylsalicyclic Acid; Amitriptyline; Ascorbic Acid; Benzoic Acid; Caffeine; Chlordiazepoxide; Chlorpromazine; Clenbuterol; Codeine; Cotinine; Dexamethasone; Dextromethorphan; Diclofenac; Dimethyl Sulfoxide; Doxepin; ε-amino-n-caproic acid; Erythromicin; Ethyl p-amino benzoate; Fenoprofen; Flunixin; Folic Acid; Folinic Acid; Furosemide; Gemfibrozil; Gentisic Acid; L-Glutamic Acid; Glycopyrrolate; Glipizide; Glutethimide; Heparin; Hippuric Acid; Hordenine; Hydrocortisone; Ibuprofen; Imipramine; Isoxsuprine; Lidocaine; Meperidine; Metaproterenol; Methadone;

Methaqualone; Methocarbamol; Methylene Blue; Methylprednisolone; Nalorphine; Naproxen; Niacinamide; Nicotine; Nortriptyline; Orphenadrine; Oxyphenbutazone; PCP; Penicillin G-Potassium; Penicillin G-Procaine; Pentoxifylline; Phenothiazine; Phenylbutazone; Polyethylene Glycol; Prednisolone; Primadone; Procainamide; Procaine; Promazine; Pyrantel; Pyrimethamine; Pyrilamine; Quinidine; Quinine; Salbutamol; Salicylamide; Salicyclic Acid; Sodium Azide; Theophylline; Thiamine; Trimethoprim; Trimipramine; Uric Acid

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.

Qualitative QC Controls: The Neogen positive and negative controls provided in the kit are for QC purposes only. The sole purpose of these controls is to verify that the test kit is performing properly. The controls are not intended for use as cutoff calibrators. The positive control is spiked at a high concentration and its approximate level can be found on the label.

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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WARRANTY

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