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CROMOGLYCATE

ELISA KIT INSTRUCTIONS PRODUCT #105819
FORENSIC USE ONLY

INTENDED USE: For the determination of trace quantities of Cromoglycate and/or other metabolites in human urine, blood, oral fluid.

DESCRIPTION

Neogen Corporation's Cromoglycate 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 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 ready-to-use 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 3 times to remove any unbound sample or drug-enzyme conjugate. The presence of bound drug-enzyme conjugate is recognized by the addition of Substrate (TMB). Optimal OD develops between 15 and 30 minutes. The test can be read visually or with a microplate reader equipped with a 650 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 blue/purple color, whereas the presence of the drug will result in light blue 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

1. **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.
2. **Drug-Enzyme Conjugate:** (ready-to-use) Do not dilute.
3. **Antibody Coated Plate:** A 96 well Nunc plate, in strips of 8 break-away wells, coated with anti-drug rabbit antiserum. The plate is ready for use as is. Do not wash.
4. **Positive Control:** 500 µL provided. Do not dilute.
5. **Negative Control:** 500 µL provided. 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. Plate cover or plastic film to cover plate during incubation.
4. Microplate reader with 650 nm filter if Red Stop is used, or a 450 nm filter if 1N HCl is used to stop the reaction. Note: It is not necessary to stop the reaction if reading immediately. Unstopped reactions should be read with a 650 nm filter.

OPTIONAL MATERIALS

1. 1N HCl or Red Stop Solution.
2. Microplate shaker.
3. EIA Buffer.

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 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.

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. **Urine:** A dilution with EIA Buffer may be necessary to reduce natural background as well as bring desired cutoff concentration within the assay range. Please contact your Neogen Representative for assistance.

b. **Whole blood:** A dilution of 1:5 (i.e. 1 part sample to 4 parts provided EIA Buffer) is recommended. Please contact your Neogen Representative for assistance.

c. **Other Forensic sample types:** Please contact your Neogen Representative for assistance.

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. Add 20 µL of sample or control to the appropriate wells in duplicate. DO NOT dilute the provided positive and negative controls.
2. Add 100 µL of ready-to-use drug-enzyme conjugate to each well. Use 8-channel pipetter or 12-channel pipetter for rapid addition.
3. Mix by gently shaking plate.
4. Cover plate with plastic film or plate cover and incubate at room temperature for 60 minutes at room temperature.
5. 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.
6. Wash each well with 300 µL of deionized water. 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 deionized water.
7. Add 100 µL of the Substrate to each well. For manual runs, use a multi-channel pipetter for best results. Mix by shaking plate gently.
8. Incubate at room temperature between 15- 30 minutes. Gently shake immediately before measuring the absorbance.

9. When Neogen's Red Stop is used, use 50 µL per well and read plate with a 650 nm filter.

SENSITIVITY

Compound	**I-50 in EIA Buffer**
Sodium Cromoglycate	9.8 ng/mL

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)**	**Sodium Cromoglycate Equivalents (ng/mL)**	**% Cross-Reactivity**
Sodium Cromoglycate	9.8	9.8	100%

Note: Sodium Cromoglycate 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%.

Acetaminophen; A.S.A; Benoxaprofen; Bumetanide; Dantrolene; Dantrolene Metabolite; Diclofenac; Ethacrynic Acid; Flunixin; Furosemide; Indomethacin; Ketoprofen; Meclofenamic Acid; Mefanamic Acid; Methysalicylate; Naproxen; Phenylbutazone; Salicylic Acid; Trichloromethiazide.

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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