Version 17-05/24

User's Manual

Mepivacaine ELISA Kit

REF **DEIA-XYL46**

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

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PRODUCT INFORMATION

Intended Use

For the determination of trace quantities of Mepivacaine and/or other metabolites in human urine, blood, oral fluid

General Description

Mepivacaine 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).

Reagents And Materials Provided

1. EIA Buffer: 40 mL (ready-to-use). Phosphate buffered saline solution with bovine serum and a preservative. Provided for dilution of drug-enzyme conjugate concentrate and 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. TMB: 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 concentrate. Dilute 180X before use.
5. Antibody Coated Plate: A 96 well Costar plate, in strips of 8 break-away wells, coated with antidrug antiserum. The plate is ready for use as is. Do not wash.
6. Stop Solution: 20 mL (ready-to-use). Non-acidic reagent used to stop the enzyme reaction.
7. Qualitative QC Positive Control: 750 µL provided (synthetic human urine). Do not dilute.
8. Qualitative QC Negative Control: 750 µL provided (synthetic human urine). Do not dilute.

Materials Required But Not Supplied

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 drug-enzyme conjugate.
6. 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

Storage

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.

Specimen Collection And Preparation

Recommended minimum sample dilutions are listed below. These dilutions may change based on your laboratory's determination. All sample dilutions should be made in our 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 our Representative for assistance.

b. Whole blood: A dilution of 1:5 (ie. 1 part sample to 4 parts provided EIA Buffer) is recommended.

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

Assay Procedure

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 180X enzyme conjugate stock 1 to 180 in the EIA Buffer provided. Mix the solution by inversion. Do not vortex. Example: for two eight well strips, add 25 µL of the 180X enzyme conjugate stock to 4475 µL of EIA Buffer.
3. Add 20 µL of sample, laboratory calibrators and Neogen controls to the appropriate wells in duplicate. DO NOT dilute positive and negative controls.
4. Add 180 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 60 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 150 µL of the TMB to each well. For manual runs, use a multi-channel pipetter for best results.
11. Incubate at room temperature for 30 minutes. Gently shake immediately before measuring the absorbance.
12. Add 50 µL of Stop Solution 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 650 nm. Wells should be read within 2 hours of stopping the reaction. Note: When Stop is used, it will result in a color ranging from a dark blue/purple to light pink based on the concentration of drug present. If 1 N HCI is preferred, use 50 µL per well and read plate with a 450 nm filter. All QC data is generated without using a stopping reagent. Note: When acid stop is used, OD values will approximately double as compared to the OD values obtained with Stop Solution.

Interpretation Of Results

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.

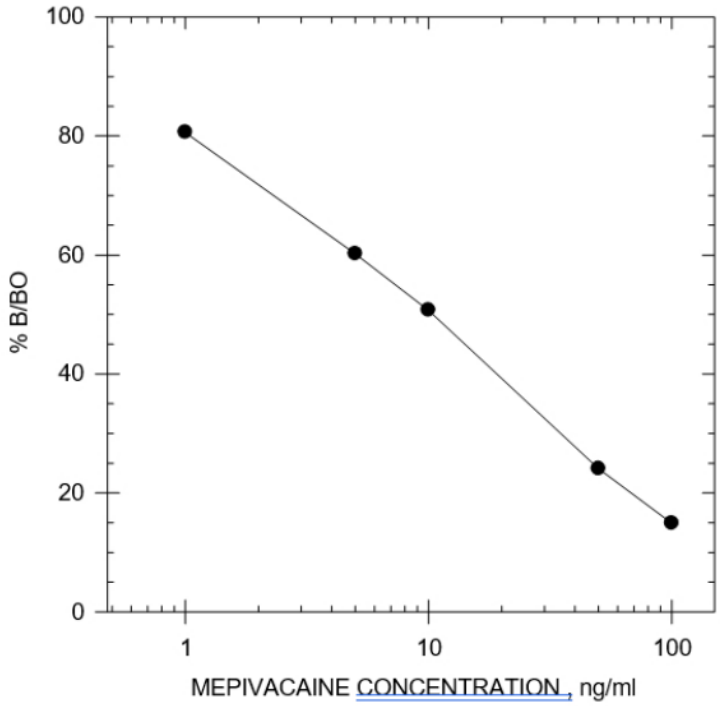
Positive Result: Samples with an absorbance less than or equal to the laboratory's designated cutoff calibrator should be considered positive. All positive samples should be confirmed by a quantitative method such as GC/MS.

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

Qualitative QC Controls: The 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.

Typical Standard Curve



Sensitivity

Mepivacaine: 5 ng/mL

Ropivacaine: 5 ng/mL

Bupivacaine: 5 ng/mL

Lidocaine: 120 ng/mL

Specificity

Compound	Compound Concentration (ng/mL)	Mepivacaine Equivalents (ng/mL)	% Cross-Reactivity
Mepivacaine	5	5	100%
Ropivacaine	5.26	5	95%
Bupivacaine	5.31	5	94%
Lidocaine	38.4	5	13%
Etidocaine	100	5	5%
Prilocaine	100	5	5%
3-Hydroxylidocaine	166	5	3%
4-Hydroxylidocaine	500	5	1%
Phenothiazine	10,000	5	0.05%
Oxyphenbutazone	25,000	5	0.02%
Methaqualone	50,000	5	0.01%

Precautions

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 TMB 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 our wash buffer.

12. Do not use Sodium Azide with samples, standards and/or calibrators.
13. Do not reuse wells, they are for one use only

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