Human Morphine Specific ELISA Kit (Competitive EIA)

Catalog number: EK7070

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

BOSTER BIOLOGICAL TECHNOLOGY
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Catalog Number: EK7070

Size: 96wells/kit

Sample Type: Serum and Plasma

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.

Kit Components

Description	Quantity
1. Microwells coated with polyclonal anti-Morphine	12x8x1
2. Morphine-Conjugate	12 ml
3. Immunalysis Positive Reference Standard	2 ml
4. Negative Standard	1 ml
5. TMB Substrate	12 ml
6. Stop Solution	11 ml

Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

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WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

1. The Morphine Specific Direct ELISA Kit is to be used with human samples, such as whole blood, oral fluids, serum, plasma and urine. Has not tested all possible applications of this assay.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2 - 40C until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (Urine samples are normally diluted 1:10 for a cutoff level of 300 ng/ml of morphine.) The dilution factor can be adjusted based on the laboratory cutoff.
2. Add 20ul. of standards into designated wells in duplicate.
3. Add 20ul. of the diluted specimens in duplicate (recommended) into designated wells.
4. Add 100ul. of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
5. Incubate for 60 minutes at room temperature preferably in the dark at room temperature (20-250C), after addition of enzyme conjugate to the last well.
6. Wash wells 6 times with 350ul distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples, containing abnormally high amounts of hemoglobin (some Postmortem samples), use 10 mM Phosphate buffered saline pH 7.0-7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.
7. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate, does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.
8. Add 100ul. of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
9. Incubate for 30 minutes at room temperature (20-250C), preferably in the dark.
10. Add 100ul. of Stop Solution to each well, to change the blue color to yellow.
11. Measure the absorbance at a dual wavelength of 450 nm. and 650 nm. Compare average absorbance readings obtained from each unknown specimen with the average absorbance obtained from the Positive Reference Standard.
12. Wells should be read within 1 hour of yellow color development.

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