BOSTER

antibody and ELISA experts

Human Opiates ELISA Kit (Competitive EIA)

Catalog number: EK7078

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.

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Catalog Number: EK7078

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
Microwells coated with polyclonal anti-morphine	12X8X1
Morphine-Conjugate	12 ml
Immunalysis Positive Ref. Std	2 ml
Neg Std	1 ml
TMB substrate	12 ml
Stop Reagent	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

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5. Absorbance paper or paper towel

6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Not for Internal or External Use in Humans or Animals. There should be no eating or drinking within work area. Always wear gloves and a protective lab coat.
4. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous. Do not add sodium azide to samples as preservative. Do not use external controls containing sodium azide. Bring all reagents to room temperature.
5. Use disposable pipet tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue. Do not pour chromogenic substrate back into container after use.
6. Do not freeze reagents. Do not mix reagents from different kit lot numbers.
7. Keep reagents out of direct sunlight. Handle stop reagent with care, since it is corrosive.
8. Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pippetting. Ensure the bag containing the micro-plate strips and dessicant is well sealed if only a few strips are used.

REAGENT PREPARATION

1. Prepare 1X wash buffer by adding the content of the bottle (25 ml, 20X) 475 of distilled or de-ionized water. Store at room temperature (20-25°C)

SPECIMEN COLLECTION AND HANDLING

1. The Opiates Direct ELISA Kit is to be used with human samples, such as urine, whole blood, oral fluids, serum and plasma. has not tested all possible applications of this assay. Cutoff criteria are important in deciding the sample dilution.
2. Specimens to which sodium azide has been added affect the assay.

STORAGE AND HANDLING

1. Urine samples should be stored at 2 -40 C 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.
2. The expiration date of the kit is stated on the label.
3. The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2-4°C.

ASSAY PROCEDURE

All reagents must be brought to room temperature (20-250C) before use. The procedure as described below may be followed in sequence using manual pipettes. Alternatively, all reagents may be added using an automated pipettor.
1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (urine samples are normally diluted 1:20 for a cutoff level of 300 ng/mL of morphine.) The dilution factor can be adjusted based on the laboratory's cutoff.
2. Add 10ul. of appropriately diluted calibrators and standards to each well in duplicate.
3. Add 10ul. of the diluted specimens in duplicate (recommended) to each well.

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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 (20-250C) preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash the 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, 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.
12. Wells should be read within 1 hour of yellow color development

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