Opiates ELISA Kit
(Catalog # E4297-100, 100 assays, Store at 4°C)

I. Introduction:
Opiates belong to the large biosynthetic group of benzylisoquinoline alkaloids, and are so named because they are naturally occurring alkaloids found in the opium poppy. The major psychoactive opiates are morphine, codeine, and thebaine. Papaverine, noscapine, and approximately 24 other alkaloids are also present in opium but have little to no effect on the human central nervous system, and as such are not considered to be opiates. Very small quantities of hydrocodone and hydromorphone are detected in assays of opium on rare occasions; it appears to be produced by the plant under circumstances and by processes which are not understood at this time and may include the action of bacteria. Dihydrocodeine, oxymorphol, oxycodone, oxymorphone, metopon and possibly other derivatives of morphine and/or hydromorphone also are found in trace amounts in opium. Despite morphine being the most medically significant opiate, larger quantities of codeine are consumed medically, most of it synthesized from morphine. Codeine has greater and more predictable oral bioavailability, making it easier to titrate the dose. Codeine also has less abuse potential than morphine, and because it is milder, larger doses of codeine are required. BioVision's Opiates ELISA kit is a competitive ELISA assay for the quantitative measurement of Opiates in serum, plasma and cell culture supernatants. The density of color is proportional to the amount of human Opiates captured from the samples.

II. Application:
This ELISA kit is used for in vitro quantitative determination of opiates class drug such as morphine, codeine, hydrocodone, hydromorphone in human samples. Detection Range: 5 - 25 ng/ml

III. Sample Type:
Human urine, whole blood, oral fluids, serum and plasma.

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4297-100</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>8 X 12 X 1</td>
<td>E4297-100-1</td>
</tr>
<tr>
<td>Opiate-Conjugate</td>
<td>12 ml</td>
<td>E4297-100-2</td>
</tr>
<tr>
<td>Immunalysis Positive Standards</td>
<td>2 ml</td>
<td>E4297-100-3</td>
</tr>
<tr>
<td>Negative Standards</td>
<td>1 ml</td>
<td>E4297-100-4</td>
</tr>
<tr>
<td>TMB substrate</td>
<td>12 ml</td>
<td>E4297-100-5</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>11 ml</td>
<td>E4297-100-6</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water and 10 mM Phosphate buffered saline (pH 7.0-7.4).
- 37°C incubator
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:
The entire kit may be stored at 4°C.

VII. Reagent and Sample Preparation:
- Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting

VIII. Assay Protocol:

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) The dilution factor can be adjusted based on the laboratory’s cutoff.
2. Add 10 µl of appropriately diluted calibrators and standards to each well in duplicate.
3. Add 10 µl of the diluted specimens in duplicate (recommended) to each well.
4. Add 100 µl 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 (20-25°C) preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash the wells 6 times with 350 µl 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.

FOR RESEARCH USE ONLY! Not to be used on humans.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com
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 100 µl 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 100 µl 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 15 minutes of yellow color development.

**IX. CALCULATION:**

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Opiates concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution. The following data represent a typical dose/response curve.

<table>
<thead>
<tr>
<th>Opiates (ng/ml)</th>
<th>450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.669</td>
</tr>
<tr>
<td>5</td>
<td>1.238</td>
</tr>
<tr>
<td>10</td>
<td>0.794</td>
</tr>
<tr>
<td>25</td>
<td>0.133</td>
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</tbody>
</table>

**X. RELATED PRODUCTS:**

- Morphine ELISA Kit (Cat. No. E4298-100)
- Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. K4256)
- Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. K4257)
- Zearalenone (ZEN) ELISA Kit (Cat. No. E4276)
- Protein Disulfide Isomerase (Opiates), human recombinant (Cat. No. 7601)
- Tamoxifen citrate (Cat. No. 1551)