DRG® Buprenorphine ELISA (EIA-1498)

Revised 20 May 2011 rm (Vers. 6.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE & SUMMARY OF TEST

The Buprenorphine ELISA is a solid phase immunoassay designed for determination of buprenorphine and nor-buprenorphin in human urine, serum and blood.
Samples are added to antibody-coated wells along with an enzyme conjugate solution. During a subsequent incubation period, the enzyme conjugate competes with any target drug in the sample for binding sites on the antibody. After a wash step to remove unbound conjugate, substrate is added, resulting in enzyme-dependent color development.
The color intensity is inversely proportional to the amount of target drug present in the sample. Thus those sample wells that contained drug will exhibit less color than the negative control well.

Summary of Drug

Buprenorphine is a semi-synthetic opioid analgesic derived from Thebaine, a component of Opium. Buprenorphine resembles morphine structurally but has both antagonists and agonist properties. Buprenorphine has a longer duration of action than morphine and is administered sublingually as an analgesic. A high dose formulation of Buprenorphine (Subutex TM) is also available as a substitution treatment of Opiate addiction. Buprenorphine has abuse potential and may itself cause dependency.

Materials Supplied

1. **Antibody Coated Microtiter Plate Wells**
   Contains twelve 8-well strips coated with high affinity antibody of interest.
2. **1 vial of 1X Buprenorphine Enzyme Conjugate (14 mL)**
   Contains the analyte of interest conjugated to horseradish peroxidase – ready to use
3. **1 bottle of 20X Wash Solution (20 mL)**
   Contains a 20x phosphate buffered saline solution with 1% Tween 20.
4. **1 bottle of Substrate (20 mL)**
   Contains a ready-to-use 3,3´,5,5´-tetramethylbenzidine solution.
5. **1 bottle of Stop Solution (20 mL)**
   Contains a 1N HCl solution.
6. **1 vial of Negative Urine Control (1 mL)**
   Contains a drug-free human urine control.
7. Instruction and Certificate of Analysis

Materials Required But Not Provided

- Positive urine control & calibrators (can be supplied upon request)
- Positive and negative serum, plasma or whole blood controls (required only if testing these sample matrices).
- Micropipettes and tips capable of delivering 10, 20, 100, 150 & 300 µL.
- Reagent reservoirs.
- Multichannel pipette or squirt bottle.
- 5 mL or 10 mL Serological pipettes.
- Microplate reader (recommended) with a 650 nm (or 450 nm filter if stopping the reaction).
- Microplate washer (optional).

Specimen Handling & Collection

Either urine, serum, plasma or whole blood can be used with this assay. Samples should be stored refrigerated. All samples materials are potentially infectious and should be handled and treated accordingly.
Anticoagulants such as heparin, citrates, oxalates and EDTA, were found not to interfere with the assay.
Specimen containing sodium azide preservative should not be tested as sodium azide can potentially interfere with the test.

Precautions

- Refrigerate contents of the kit at 2 °C – 8 °C when not in use. DO NOT FREEZE.
- Prior to performing the test, allow all kit components, controls, and samples to reach room temperature (20 °C – 23 °C).
- For the ready-to-use Enzyme Conjugate, only allow sufficient volume for number of tests being run to reach room temperature i.e. 1 mL is sufficient for 8 tests. Stock solution must be returned to the refrigerator immediately.
- Use separate disposable pipette tips for each sample and control to avoid cross-contamination.
- Do not mix components from different kits.
- Discard used material in proper disposal containers.
- Do not use reagents beyond their expiration dates.
- Urine samples with very low or very high pH (< 4 or > 10) may interfere with ELISA based tests. Very acidic or basic samples must be normalised within this range.

Wash Solution Preparation

Prepare Wash Solution by diluting contents of the 20X Wash Solution bottle (20 mL) into 380 mL laboratory grade water. Swirl to mix.

Assay Procedure

Note: It is recommended to run sample and controls in duplicate in order to optimize accuracy. Duplicates should be run in adjacent wells.

1. Add **20 µL** of the controls directly to the bottom of a designated well(s).
2. Add **20 µL** of each sample directly to the bottom of designated well(s).
3. Add **100 µL** of the Buprenorphine Enzyme Conjugate solution to each well. Mix by tapping the sides of the strip holder 3 or 4 times.
4. Allow the reaction to incubate at room temperature for **30 minutes**. Mix by tapping the side of the strip holder 3 or 4 times.
5. Dump" the solution from the wells into an appropriate waste container. Using a multichannel pipette, add ≥ 300 µL of wash solution (prepared as described above) to each well, or fill to overflowing with a squirt bottle. Dump the Wash Solution and while inverted, sharply strike against absorbent paper 3 or 4 times to remove excess moisture. Repeat the wash step 4 more times. Ensure that the wells are free of droplets or bubbles after the final wash. If bubbles are present, use a clean pipette tip to break them. After breaking the bubbles, or if droplets are present, tamp one additional time.
   An automated washer can be used to wash the microplate. A typical wash cycle will include: aspiration followed by wash. Repeat 3-4 times. Adjust volume to 350 µL and ensure that a crosswise aspiration is used. This provides optimal washing.
6. Proceed immediately to Substrate addition. Do not allow the wells to dry out. Add **150 µL** of Substrate to each well. Mix by tapping the side of the strip holder 3 or 4 times.
7. Allow the reaction to incubate at room temperature for **10-15 minutes** or until a medium blue color appears in the negative control wells. Mix by tapping 2 or 3 times during color development.
8. Read the results with a microplate reader and a 650 nm filter. If no microplate reader is available, visually compare unknown samples to the color in the negative and positive control wells.

Or

Optional Step: Stop the reaction by adding **150 µL** of Stop Solution to each well. This will turn any wells exhibiting a blue color to yellow. Using a microplate reader and a 450 nm filter, blank the reader on air and read the optical density of each well.

Quality Control & Calibration

Good laboratory practice suggests the use of controls to ensure proper assay performance.
For semi-quantitative analysis, calibrators are available and sold separately.