



## OXYCODONE RIA KIT

Cat: 117-0100

Cat: 117-2200

### Details of the Procedure.

All reagents must be brought to room temperature before use. The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added simultaneously using an automated pipettor

1. Set up and label as many duplicate tubes as are required for the Positive Reference Standards, the Negative Standard and the urine specimens to be assayed.
2. Add 10  $\mu$ l of Positive Reference Standards and Normal Control to the appropriate tubes.
3. Add 10  $\mu$ l of each urine specimen to the appropriate tubes.
4. Add 200  $\mu$ l of  $^{125}$ I- Oxycodone Reagent (Colored green) to each tube.
5. Add 100  $\mu$ l of blue Anti-Oxycodone specific serum (Colored blue) Reagent to each tube; mix well on a vortex-type mixer.
6. Add 200  $\mu$ l of Goat anti Rabbit Second Antibody Reagent (**shake well before use**) to each tube. (colored orange) **CAUTION: DO NOT MIX THIS SECOND ANTIBODY REAGENT WITH THE SECOND ANTIBODY FROM THE OTHER RIA KITS**
7. Gently vortex mix all tubes and incubate for 60 minutes or any interval up to 3 hours at room temperature (25°C). Standards, samples and controls must be incubated together for the same time period. The assay rack may be covered with parafilm.
8. Centrifuge the tubes for 10 minutes, at approximately 1200- 2500 x g in a swinging bucket rotor, or at least 3500-4000g in a fixed angle head rotor. **Centrifugation time may be extended, if necessary, to optimize formation of suitable pellets.**
9. Decant supernatant, drain (optional) and blot each tube.
10. Count each tube in a gamma scintillation counter to obtain counts per minute (CPM).
11. Compare average counts per minute obtained from each unknown specimen with the average CPM obtained from the Positive Reference Standard.

### SAMPLE DOSE RESPONSE CURVE

Oxycodone ng/ml	CPM
0	122245
300	70108
600	55857
1000	45577
5000	19597

## Cross Reactivities with Related Drugs

The following compounds were run at 10,000 ng/ml and the response read of the dose response curve

Compound	Equivalents of Oxycodone ng/ml	Cross-reactivity
Morphine	18	0.18
Codeine	69	0.69
Morphine 3-gluc.	16	0.16
6-acetyl-codeine	134	1.34
Hydromorphone	138	1.38
Hydrocodone	339	3.39
Norcodeine	27	0.27
Normorpheine	<5.0	<0.05
Noroxycodone	91	0.91
Noroxymorphone	25	0.25
Dihydrocodeine	126	1.26
Oxymorphone	1559	15.59

## Cross-Reactivities with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 50,000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level ( 5 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid Atropine , Barbitol, Benzoyllecgonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Chloroquine , Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene , 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital , Mephenytoin,  $\alpha$ -Methyl- $\alpha$ -propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide , 4-Methylprimidone, Meperidine , Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital , Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH