



# AMPHETAMINE DIRECT RIA KIT [I-125]

## Immunalysis Corporation:

Catalog Number 109-0100 100 tubes

Catalog Number 109-0500 500 tubes

## INTRODUCTION

A radioimmunoassay (RIA) for amphetamine in urine is described. A 50 ul aliquot of sample is incubated with a 100 ul dilution of sheep anti-amphetamine antibody (polyclonal) and 200 ul of I-125-amphetamine reagent. Separation of the bound I-125- amphetamine is by a second antibody complex. The technique is sensitive to 5 ng/ml without dilution or other manipulation. The National Institute on Drug Abuse recommends a 1000 ng/ml cut-off for amphetamine.

## INTENDED USE

The RIA Kit [I-125] is intended for detection and semi-quantitation of amphetamine in urine at 1000 ng/ml or higher.

**The Immunalysis Amphetamine Direct RIA Kit [I-125] provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC-MS) is the preferred confirmatory method (1). Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

## EXPLANATION OF THE TEST

The Immunalysis Amphetamine Direct RIA Kit is a specific and sensitive in-vitro test for amphetamine in urine. Interference by l-methamphetamine and pseudo-ephedrine is virtually nonexistent. Amphetamine is a potent central nervous system stimulant. The (+)-isomer also referred to as d-amphetamine is three to four times more potent than the (-)-isomer, l-amphetamine (2). Amphetamine may be metabolized and excreted as the p-hydroxy isomer. However up to 80% of a given dose may be excreted unchanged, especially in acid urine

The Immunalysis Amphetamine Direct RIA Kit [I-125] provides a positive reference standards and a normal reference control, I-125 amphetamine radioligand, amphetamine antibody and a second antibody complex to precipitate antibody-bound amphetamine.

## PRINCIPLES OF THE PROCEDURE

The Immunalysis Amphetamine Direct RIA Kit [I-125] is based upon the competitive binding to antibody of I-125 radiolabeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture (4). An unknown specimen is mixed in a test tube with fixed amounts of sheep anti-amphetamine antibody (polyclonal) and radiolabeled antigen. Antigen present in a patient sample competes with

labeled antigen for the limited antibody present. After precipitation of the antigen-antibody complex with a second antibody reagent and centrifugation, the tubes are aspirated or decanted and the pellets containing bound antigen are counted in a gamma scintillation counter. Sample counts per minute (CPM) values equal to or less than the CPM value of the cut-off reference standard (500 ng/ml amphetamine) are indicative of the presence of amphetamine in the urine specimen.

The Immunalysis Amphetamine Direct RIA Kit [I-125] employs an amphetamine directed antiserum. There is little or no interference with binding proteins(s) or other macromolecules.

## MATERIALS AND METHODS

Materials and equipment required but not supplied with the Immunalysis Amphetamine Direct RIA Kit [I-125] are itemized below:

### Materials

- 12x75 mm Disposable Glass or Plastic Culture Tubes.
- Test Tube Racks.
- Manual micropipets or automated pipetting stations

### Equipment

- Refrigerator (for kit storage).
- Vortex Mixer.
- Interval Timer.
- Centrifuge.
- Gamma Counter calibrated for <sup>125</sup>I.
- Calculator.

## REAGENTS

### Immunalysis Amphetamine Direct RIA Kit I-125 Contents.

Each Immunalysis Amphetamine Direct RIA Kit [I-125] contains:

Contents	100 Test Kit*
1 bottle amphetamine Antibody	11 ml
1 bottle I-125-amphetamine (not more than 10 uCi)	22 ml
1 bottle containing 1000 ng/ml amphetamine in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine)	4 ml
1 bottle Second Antibody-PEG Complex	22 ml

Amphetamine Antibody. The antibody solution is anti-amphetamine serum diluted in 0.1 M phosphate buffer, pH 7.0, with BSA 0.1%, sodium chloride 0.9%, and sodium azide 0.1% (colored blue).

I-125-amphetamine. The tracer solution contains not more than 250 uCi of I-125-amphetamine in 0.1 M phosphate buffer, pH 7.0, with BSA (0.1%), sodium chloride (0.9%) and sodium azide (0.1%) (colored green).

Positive Reference Standards. These bottles contain varying amounts of amphetamine dissolved in a human urine matrix: 250, 500, and 1000 ng/ml.

Normal Control. This bottle contains a drug free human urine matrix.

Second Antibody-PEG Complex. The separation reagent contains a second antibody-PEG complex in 0.1 M phosphate buffered saline (colored pink).

#### Precautions

For In Vitro Diagnostic Use.

Not for Internal or External Use in Humans or Animals.

Radioactive Warning. This radioactive material may be received, acquired, possessed and used by physicians, clinical laboratories and hospitals laboratories possessing a specific license, and only for in-vitro clinical or laboratory tests not involving internal or external administration of the material or the radiation therefrom to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of the State with which the Commission has entered into an agreement for the exercise of regulatory authority.

This kit contains radioactive material which should be handled according to the following guidelines:

- (1) All radioactive materials should be stored and used in specially designated areas.
- (2) No pipetting should be done by mouth.
- (3) There should be no smoking or eating within the work area.
- (4) Hands should be washed after using radioactive materials.
- (5) Work should be carried out on a surface covered with absorbent materials.
- (6) Any spills of radioactive material should be cleaned immediately and all contaminated materials disposed of as radioactive waste. Contaminated surfaces should be washed with a detergent.

Sodium Azide Hazard. Sodium azide can violently react with the copper, lead, brass or solder in plumbing systems. Since sodium azide has been added in a concentration of 0.1% to the buffer, standards and antibody, these reagents should be disposed into the drain system together with copious amounts of water. Copper-free and lead-free plumbing is recommended.

General. Precise pipetting is the essence of successful radio immunoassay. Micropipets supplied by "Eppendorf" or "SMI" with disposable tips are excellent when used carefully according to instructions to insure the necessary accuracy. New automatic dispensers improve reliable delivery.

Storage. The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2 - 4 degrees centigrade.

Treatment Required. There is no sample treatment or reagent purification required for the use of the Immunalysis Amphetamine Direct RIA Kit I-125.

Indications of Deterioration. A drop of greater than 25% in the zero-dose (maximum bound %) for a constant incubation time indicates deterioration of the antibody or tracer. A significant shift of the standard curve to the right would result from deterioration of the standards. Non-specific binding above 15% indicates deterioration of the tracer.

#### SPECIMEN COLLECTION

#### Precautions.

The Immunalysis Amphetamine Direct RIA Kit [I-125] is to be used with human urine samples.

Additives. Urine specimens to which sodium fluoride has been added or preservatives necessary to prevent bacterial growth do not affect the assay.

Interfering Substances. There are no commonly abused drugs which alter the values obtained with the Immunalysis Amphetamine Direct RIA Kit [I-125]. Samples containing radioactive contamination from previous in vivo diagnostic procedures should be rejected.

Storage and Handling Instructions. Urine samples should be stored at 2 - 4 degrees centigrade 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.

#### Details of the Procedure.

All reagents must be brought to room temperature before use.

1. Set up and label as many duplicate tubes as are required for the Positive Reference Standards, the Normal Control and the urine specimens to be assayed.
2. Add 50 ul of Positive Reference Standard and Normal Control to the appropriate tubes.
3. Add 50 ul of each urine specimen to the appropriate tubes.
4. Add 200 ul of I-125 amphetamine to each tube.
5. Add 100 ul of blue Anti-amphetamine Serum Reagent to each tube.
6. Add 200 ul of Second Antibody Reagent (**shake well before use**) to each tube.
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 1000 ng/ml Positive Reference Standard.

It is recommended that at least one in-house positive quality control sample be included with every assay run. Should you desire to determine NSB, use 100ul of Normal control in place of antibody.

Sodium Periodate (50 ul) should be added to all tubes if interference from ephedrine or pseudoephedrine is suspected.

#### RESULTS

If the average sample CPM is equal to or less than the average CPM of the Positive Reference Standard the sample is POSITIVE for amphetamine (has an amphetamine concentration equal to or greater than 1000 ng/ml). If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for amphetamine (less than 1000 ng/ml amphetamine).

Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding CPM (ordinate). Values for unknown samples are obtained by interpolation from the curve.

THE FOLLOWING DATA REPRESENT A TYPICAL DOSE/RESPONSE CURVE.

d-amphetamine ng/ml	Mean CPM
0	81520
250	44973
500	33245
1000	24417

The dose/response curve shown above is an example run and should not be used for assay calculations. The curve generated at time of assay is suitable to calculate the concentration of drug in sample. The dose/response plot is sharp and linear from the low point through the 1000 ng/ml cut-off up to the high point on the plot.

#### SPECIFIC PERFORMANCE CHARACTERISTICS

##### Accuracy

Urine samples (50) collected from presumed non-users were tested in the Immunalysis Amphetamine Direct RIA Kit [I-125]. One hundred percent of these normal urine samples measured negative at 500 ng/ml. Random RIA positive urine samples (53) were subjected to GC/MS analyses. All samples were confirmed as true positives.

##### Precision

The precision of the Immunalysis Urine Amphetamine Direct RIA Kit [I-125] has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.

##### Intra-assay Precision

Intra-assay precision was determined with reference controls. A 250, 500, and 1000 ng/ml standard was assayed five times in the same assay. The results are tabulated in Table 1.

Table 1

Amphetamine (ng/ml)	Mean CPM	S.D.	C.V.%
250	42749	1229	2.87
500	32516	906	2.79
1000	24654	874	3.5

Intra-assay CVs for Reference Controls ranged from 2.87 to 3.5%

##### Inter-assay Precision

Inter-assay precision was examined using reference controls. A 250, 500 and 1000 ng/ml reference control was assayed in ten separate runs over a 24 hour period and a SD and CV determined. Results are tabulated in Table 2.

Table 2

Inter-assay Precision (Reference controls)				
ng/ml Amphetamine	No of determinations	Mean CPM	S.D	C.V.%
250	10	42588	507	1.2
500	10	33760	599	1.8
1000	10	25239	634	2.5

Reference Control inter-assay CVs ranged from 1.2 to 2.5%. The average CV was 1.83%.

##### Sensitivity

Assay sensitivity based on the minimum amphetamine concentration required to produce a four standard deviation from assay Bo is 5 ng/ml.

The National Institute on Drug Abuse recommends a 1000 ng/ml cut-off for amphetamine.

##### Specificity

The following compounds were tested for cross-reactivity in the Immunalysis Amphetamine Direct RIA Kit [I-125]. The compounds tested were prepared in a human urine matrix. The results are expressed as the value obtained when the compound was tested at two levels.

Table 3

Compound	Approx. ng/ml equivalent to 500ng amphetamine	Cross-reactivities at 50% Inhibition
I-Amphetamine	2630	19
Hydroxyamphetamine HCl	247	40
I-Methamphetamine HCl	25400	2
d-Methamphetamine HCl	4541	11
d-Phenylpropanolamine	>50000	<1
I-Phenylpropanolamine	>50000	<1
d-Pseudoephedrine	>50000	<1
I-Pseudoephedrine	>50000	<1
MDMA.HCl	>50000	<1
Tyramine	>50000	<1

### Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 20,000 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, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbitol, Benzoyllecgonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Codeine, 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, Methbarbital, Mepherytoin, "-Methyl"-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH

### Recovery

Aliquots of a human urine matrix were spiked with amphetamine to give a final theoretical concentration of 250, 500 and 1000 ng/ml. Each of these controls were assayed in replicates of 10 within a test run, and the subsequent experimental concentration and recovery calculated. The results are tabulated in Table 4.

Table 4  
Recovery

Spiked urine Concentration (ng/ml)	Observed amphetamine conc. (ng/ml)	% Recovered
250	249.2	99.9
500	497.7	99.5
1000	997.2	99.7

### Interfering Substances

There are no known interfering substances which alter the values obtained with the Immunalysis Amphetamine Direct RIA Kit (I-125).

### Expected Values

The urine level of amphetamine in humans who have not used amphetamine within the last two weeks is usually negative, i.e. below 1000 ng/ml by the Immunalysis Amphetamine Direct RIA Kit (I-125) (1,5). User levels range from 500 to 1000+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine amphetamine level depends on the individual metabolism, the amount and purity of the amphetamine used and the time elapsed since last use.

### Interpretation

Amphetamine appears in the urine within hours after drug use and may persist for days (1,5). The Immunalysis single step, single incubation assay is characterized by a sharp linear plot through the 500 ng/ml cut-off from the low point to the high point.

### Limitations of the Procedure

Samples containing radioactive contamination from previous in-vivo diagnostic procedures should be rejected. Clinical consideration and professional judgement should be applied to any drug abuse test, particularly when preliminary positive results are used. There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results e.g. technical or procedural errors.

### REFERENCES

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4. R. S. Yallow and S. A. Berson. Nature. 184: 1648 - 1649 (1959).
5. R. C. Baselt. In: Advances in Analytical Toxicology, Vol.1. p.87 - 93. Ed. R. C. Baselt, Biomedical Publications, Foster City, CA (1984).

This package conforms to the conditions and limitations specified in 49 CFR 173.421 for radioactive material, excepted package - limited quantity of material, UN2910.

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