



# THC-CANNABINOIDS DIRECT RIA KIT [<sup>125</sup>I]

## Immunalysis Corporation:

Catalog Number 105-0100 100 tubes

Catalog Number 105-0500 500 tubes

## INTRODUCTION

A radioimmunoassay (RIA) for urine cannabinoids is described. The assay involves a single incubation of specimen (50 ul) with a 100 ul dilution of sheep anti-cannabinoid (serum) antibody and 200 ul of <sup>125</sup>I -cannabinoid reagent. Separation of the unbound <sup>125</sup>I -THC is by use of a second antibody-PEG complex. The assay features a 100 ng/ml cut-off but is sensitive to 2.5 ng/ml without dilution or other manipulation.

## INTENDED USE

The Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] is intended for detection and semi-quantitation of cannabinoids in urine at or above 50 ng/ml. **The Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] 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

Δ<sup>9</sup>-THC (a member of the cannabinoid family) is the primary psychoactive ingredient of marijuana (1). Cannabinoid metabolites appear in urine two to four hours after a marijuana smoke and may persist for days (up to thirty)(1- 3). Thus a urine assay reasonably serves to detect cannabis use even though a considerable period may have elapsed since smoking or ingestion of marijuana.

The Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] provides a positive reference standard of 100 ng/ml of 11-nor-9-carboxy-Δ<sup>9</sup>-THC (CTHC) and a normal reference control, I-125-THC reagent, cannabinoid antibody and a second antibody-PEG complex to precipitate THC bound to antibody.

## PRINCIPLES OF THE PROCEDURE

The Immunalysis Urine THC-Cannabinoids Direct RIA Kit [I-125] for cannabinoids is based upon the competitive binding to antibody of I-125 radiolabeled antigen and unlabeled antigen, in proportion to their concentrations in the reaction mixture.

An unknown specimen is mixed in a test tube with fixed amounts of sheep anti-cannabinoid 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 CPM values equal to or less than the CPM value of the cut-off (100 ng/ml) CTHC standard are indicative of the presence of cannabinoids/cannabinoid metabolites in the urine specimen.

The Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] involves only one 60 minute incubation and avoids extraction of urine sample for measurement. There is little or no interference with binding protein(s) or other macromolecules.

## MATERIALS AND METHODS

Materials and equipment required but not supplied with the Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] are itemized below:

### Materials

- 12x75 mm Disposable Glass or Plastic Culture Tubes.
- Test Tube Racks.
- 0.05, 0.2 ml and 0.5 ml micropipets.

### Equipment

- Refrigerator (for kit storage).
- Vortex Mixer.
- Interval Timer.
- Centrifuge.
- Gamma Counter.
- Calculator.

## REAGENTS

Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] Contents.  
Each Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] contains:

Contents	100 Test Kit
1 bottle Cannabinoid Antibody	11 ml
1 bottle <sup>125</sup> I -THC reagent	21 ml
1 bottles containing 100ng/ml CTHC in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine )	4 ml
1 Bottle Second Antibody-PEG Complex	21 ml

Cannabinoid Antibody. The antibody solution is sheep anti-cannabinoid 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).

<sup>125</sup>I-THC Reagent. The tracer solution contains <sup>125</sup>I-THC 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 Standard. This contains 100 ng/ml of 11-nor-9-carboxy-delta-9-THC dissolved in a synthetic urine matrix.

Normal Control. This contains a drug free synthetic 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 ensure 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 °C.

**Treatment Required.** There is no sample treatment or reagent purification required for the use of the Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I].

**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 25% indicates deterioration of the tracer.

**SPECIMEN COLLECTION**

**Precautions:** The Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] 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 THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I]. 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° 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.

**DETAILS OF THE PROCEDURE.**

All reagents must be brought to room temperature before use. The procedure as described below should 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 tubes as you require for the Positive Reference Standard, the Normal Control and the urine specimens to be assayed.
2. Add 50 ul of each Reference Standard, Normal Control to the appropriate tubes.

3. Add 50 ul of each urine specimen to the appropriate tubes.
4. Add 200 ul of green <sup>125</sup>I Cannabinoid Reagent to each tube.
5. Add 100 ul of blue anti-Cannabinoid Serum Reagent to each tube; mix well on a vortex-type mixer.
6. Add 200 ul of pink 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 15 minutes, at approximately 1200-2500 g in a swinging bucket rotor, or 3500-4000g in a fixed angle head rotor. **Centrifugation time may be extended, if necessary, to optimize formation of suitable pellets.**
9. Decant/aspirate supernatant immediately after centrifugation. Then 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 100ng/ml Positive Reference Standard.

It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve can be established by preparing dilutions of the High Control with the normal reference control. Should you desire to determine NSB, use 100ul of Normal control in place of antibody.

**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 cannabinoids (has a cannabinoid concentration equal to or greater than 100 ng/ml). If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for cannabinoids (less than 100ng/ml cannabinoids).

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. Note that the entire clinical range is perfectly linear without manipulation.

CTHC ng/ml	Mean CPM
0	92133
25	68130
50	51323
100	39081

The dose/response curve shown above should not be used in assay calculations. A dose/response curve generated at time of assay is suitable for calculation of drug concentration in sample. The dose/response plot is sharp and linear from the low point through the 100 ng/ml cut-off to the high point.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

Accuracy :

Urine samples (40) collected from presumed non-users were tested in the Immunalysis Radioimmunoassay for cannabinoids. 100 % of these normal urines measured negative at 100 ng/ml. Random RIA positive urine samples (53) were subjected to GC-MS analyses. All samples were confirmed as true positive.

Precision

The precision of the Immunalysis Urine Direct RIA Kit [<sup>125</sup>I] has been verified by the assessment of the mean, standard deviation

(SD) and coefficient of variation (CV) in data resulting from repetitive assays.

Intra-assay Precision

Intra-assay precision was determined with both patient samples and reference controls.

Intra-assay precisions (Patient samples).

A low, mid, and high range patient sample was assayed five times in the same assay. The results are tabulated in Table 1.

TABLE 1

Intra-assay Precision				
11-nor- $\Delta^9$ -THC-9				
Carboxylic Acid counts (ng/ml)	counts per min.	S.D.	C.V.%	
<b>Low Range</b>				
25	86550			
27	87120	86754	814.2	0.94
29	85990			
26	86130			
25	87980			
<b>Mid Range</b>				
57	77770			
63	75870	76108	1354.8	1.78
62	74100			
59	76800			
60	76000			
<b>High Range</b>				
105	68970			
102	69340	68270	1566.3	2.29
107	67020			
111	66210			
101	69810			

Intra-assay CV of low, mid and high range patient samples are consistently below 2.5%. The CV around assay cut-off was 1.78%.

Intra-assay Precision (Reference Controls)

A 25, 50, and 100 ng/ml standard was assayed five times in the same assay. The results are tabulated in Table 2.

TABLE 2

Intra-assay Precision (reference Controls)

11-nor- $\Delta^9$ -THC-9 x			
Carboxylic Acid (ng/ml)	counts per min.	S.D.	C.V.%
25	86550	1461	1.7
50	72730	1523	2.1
100	64060	604	0.94

Intra-assay CVs for Reference Controls ranged from 0.94 to 2.1%.

Inter-assay Precision Inter-assay precision was determined using both patient samples and reference controls.

Inter-assay Precision (Patient samples)

A low, mid, and high range patient sample was assayed in ten separate runs to determine the inter-assay precision. Results are tabulated in Table 3.

TABLE 3  
Inter-assay precision ( Patient samples)

Patient sample	No of deter-minations	Mean conc. ng/ml per min.	Mean count	S.D.	C.V.%
Low range	10	27	87530	767	0.87
Mid range	10	58	76820	613	0.80
High range	10	110	67100	359	0.54

Average C.V. = 0.74

Patient sample inter-assay CVs ranged from 0.54 to 0.87%.

Inter-assay Precision (Reference controls)

A 25, 50, and 100 ng/ml reference control was assayed in ten separate runs and a S.D. and CV determined. Results are tabulated in Table 4.

TABLE 4  
Inter-assay Precision (Reference controls)

Patient sample	No of deter-minations	Mean conc. ng/ml per min.	Mean count	S.D.	C.V.%
25	10	24	84510	1411	1.67
50	10	53	70130	1320	1.88
100	10	101	65010	861	1.32

Reference control inter-assay CVs ranged from 1.32 to 1.88%. The average CV was 1.62%.

Sensitivity

Assay sensitivity based on the minimum cannabinoid concentrations required to produce a four standard deviation from assay Bo is 2.5 ng/ml. A conservative 100 ng/ml cut off is recommended.

Recovery

Aliquots of a human urine matrix were spiked with 11-nor-9-carboxy- $\Delta^9$ -THC to give a final theoretical concentration of 25, 50, and 100 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 5.

TABLE 5  
9CTHC Recovery in ng/ml

Conc.	Mean Observed Concentration	X% of spike Recovery-9CTHC
25	24	96.0
50	49.3	98.6
100	98	98.0

Specificity

The specificity of the Immunalysis Radioimmunoassay for

cannabinoids [<sup>125</sup>I] for various cannabinoids and cannabinoid metabolites was determined by generating inhibition curves for each of the compounds and then determining by extrapolation the percentage cross-reactivity at 50 percent B/Bo.

The antisera cross-reactivities are listed in Table 6.

TABLE 6  
Cross Reactivities

Compound	Approx. ng/ml equivalent to 100 ng 11-nor-9-carboxy- $\Delta^9$ -THC/ml	Cross-reactivities at 50% Inhibition (%)
11-nor-9-carboxy- $\Delta^9$ -THC	108	108
11-hydroxy- $\Delta^9$ -THC	>10000	<5
8-11-Dihydroxy $\Delta^9$ -THC	>10000	<5
$\Delta^9$ -THC	>10000	<5
Cannabinol	>10000	<5
Cannabidiol	>10000	<5

**Cross-Reactivity with Unrelated Drugs**

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 10,000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (2.5 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbital, 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, Methamphetamine, Metharbital, Mephentoin,  $\Delta$ -Methyl- $\Delta$ -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.

**Expected Values**

The urine level of CTHC in humans who have not used marijuana within the last three weeks will be negative, reading below 50 ng/ml by the Immunalysis THC-Cannabinoids Direct RIA Kit [<sup>125</sup>I] (4). After a marijuana cigarette the peak urine concentration occurs 2 to 4 hours following the smoke (1-3). The absolute urine levels of cannabinoid depends on the individual subject's metabolism, as well as the amount and purity of the marijuana smoked or ingested.

Passive inhalation under extreme conditions has been reported to result in urine cannabinoid levels above 50 ng/ml (6). However, realistic situations have never produced levels greater than 20 ng/ml (4).

**Interpretation**

Cannabinoids appear in urine several hours after marijuana use and persist from 7 - 10 days to as long as 30 days (1-3). The Immunalysis highly sensitive (2.5 ng/ml) single step, single incubation assay characterized by a sharp and linear plot through the 50 ng/ml cut-off from the low point through the high con-

centration point facilitates reliable detection of marijuana in urine. It should be noted that a positive urine cannabinoid level does not correlate with recent exposure to marijuana.

**LIMITATIONS OF THE PROCEDURE**

Samples containing radioactive contamination from previous in vivo diagnostic procedures should be rejected.

The Immunalysis Urine THC-Cannabinoids 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. Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used. There is the 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**

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph . 73, 1986.
2. Rodgers, R., Crowl C.P., Eimstad W.M., et al.: Homogeneous enzyme immunoassay for cannabinoids in urine. Clin. Chem. 24: 95 (1978).
3. Teale, J.D., Foxman, E.J., King, L.S., Pial, E.M. and Marks, V.: The development of a radioimmunoassay for cannabinoids in blood and urine. J. Pharm. Pharmacol. 27: 465 (1975).
4. Mule, S.J., Lomax, P. and Gross, S.J.: Active and passive marijuana exposure tested by three immunoassays and GC/MS in urine. J. Anal. Toxicol. 12: 113 (1988).
5. Frederick, D.L., Green, J. and Fowler, M.W.: Comparison of six cannabinoid metabolite assays. J. Anal. Toxicol. 9: 116 (1985).
6. Cone, E.J. and Johnson, R.E.: Contact highs and urinary cannabinoid excretion after passive exposure to marijuana smoke. Clin. Pharmacol. Ther. 40: 247 (1986).

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

**Immunalysis Corporation  
Pomona, CA 91767  
June 2001**