

# One Step Drug of Abuse Test

(Dip Card)

Package Insert for Kratom Screen Test

This instruction sheet is for the testing of Kratom (KRA) drug. A rapid, one step screening for the qualitative detection of Kratom and its metabolites in human urine.

## For Forensic Use Only

### INTENDED USE

The **One Step Drug of Abuse Test** is a lateral flow chromatographic immunoassay for the qualitative detection of Kratom and its metabolite in urine at the following cutoff concentration:

Test	Calibrator	Cut-off
Kratom (KRA)	Mitragynine	100ng/mL

This assay provides only a preliminary qualitative test result. Use a more specific alternate quantitative analytical method to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.<sup>1</sup> Apply clinical and professional judgment to any drug of abuse test result, particularly when preliminary positive results are obtained.

### SUMMARY AND EXPLANATION OF THE TEST

The **One Step Drug of Abuse Test** is a competitive immunoassay utilizing highly specific reactions between antibodies and antigens for the detection of multiple drugs and drug metabolites in human urine with the use of an instrument.

#### KRATOM (KRA)

Kratom is from a leaf of the kratom plant, it's a natural narcotic produced mainly in southern Thailand, containing Mitragynine and 7-Hydroxymitragynine. It is often used to relieve chronic diseases, alcohol, etc., and relieve the pain caused by withdrawal of opium. A pain-eating cocktail made from the pain bearing leaves is the most common and readily available drug drink in southern Thailand. It can cause drowsiness and paralysis.

The KRA assay contained within the **One Step Drug of Abuse Test** yields a positive result with the concentration of Mitragynine in urine exceeds 100ng/mL.

### PRINCIPLE

The **One Step Drug of Abuse Test** is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against its respective drug conjugate for binding sites on their specific antibody.

During testing, a urine specimen migrates upward by capillary action. A drug, if present in the urine specimen below its cut-off concentration, will not saturate the binding sites of its specific antibody. The antibody will then react with the drug-protein conjugate and a visible colored line will show up in the test line region of the specific drug dip card. The presence of drug above the cut-off concentration will saturate all the binding sites of the antibody. Therefore, the colored line will not form in the test line region.

A drug-positive urine specimen will not generate a colored line in the specific test line region of the dip card because of drug competition, while a drug-negative urine specimen will generate a line in the test line region because of the absence of drug competition.

To serve as a procedural control, a colored line will always appear at the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

### REAGENTS

The test contains a membrane dip card coated with drug-protein conjugates (purified bovine albumin) on the test line, a goat polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with mouse monoclonal antibody specific to kratom drug.

### PRECAUTIONS

- For Forensic Use Only.
- Do not use after the expiration date.
- The test dip card should remain in the sealed pouch until use.
- The test is for single use only.
- While urine is not classified by OSHA or the CDC as biological hazard unless visibly contaminated with blood<sup>2,3</sup>, the use of gloves is recommended to avoid unnecessary contact with the specimen.
- The used test dip card and urine specimen should be discarded according to federal, state, and local regulations.

### STORAGE AND STABILITY

Store as packaged in sealed pouch at 4-30°C (39-86°F). The test is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

## SPECIMEN COLLECTION AND PREPARATION

#### Urine Assay

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be allowed to settle to obtain a clear specimen for testing.

## MATERIALS

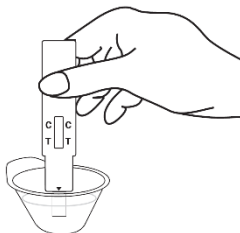
#### Materials Provided

- Test dip card
- Desiccants
- Package insert
- Materials Required But Not Provided
- Specimen collection container
- Disposable gloves
- Timer

## DIRECTIONS FOR USE

Allow the test dip card, and urine specimen to come to room temperature [15-30°C (59-86°F)] prior to testing.

- Remove the dip card from the foil pouch. Remove the cap from the test dip card. Label the dip card with patient or control identifications.
- Immerse the absorbent tip into the urine sample for 1 minute. Urine sample should not touch the plastic device.
- Replace the cap over the absorbent tip and lay the dip card flatly on a non-absorbent clean surface.
- Read result at 5 minutes. **DO NOT READ RESULT AFTER 5 MINUTES.** (Fig. 1)



(Fig. 1)

## INTERPRETATION OF RESULTS

(Please refer to the illustration below)



**NEGATIVE:** Two lines appears.\* One color line should be in the control region (C), and another apparent color line adjacent should be in the test region (T). This negative result indicates that the drug concentration is below the detectable level.

\*NOTE: The shade of color in the test line region (T) will vary, but it should be considered negative whenever there is even a faint distinguishable color line.

**POSITIVE:** One color line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the drug concentration is above the detectable level.

**INVALID:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failures. Review the procedure and repeat the test using a new test dip card. If the problem persists, discontinue using the lot immediately and contact your supplier.

## QUALITY CONTROL

A procedural control is included in the test. A color line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

## LIMITATIONS

- The **One Step Drug of Abuse Test** provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.<sup>4,5,6</sup>
- There is a possibility that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen and a new test dip card.
- A positive result does not indicate intoxication of the donor, the concentration of the drug in the urine, or the route of drug administration.
- A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
- Test does not distinguish between drugs of abuse and certain medications.
- A positive test result may be obtained from certain foods or food supplements.

## PERFORMANCE CHARACTERISTICS

#### Accuracy

In the comparison study, the **One Step Drug of Abuse Test** was compared to a GC/MS reference method to determine its accuracy. Clinical urine samples were collected for Kratom listed on the follow table. Clinical specimens were quantified by GC/MS analysis before testing.

Test	Compound Contributed to the Total of GC/MS
KRA	Mitragynine

The following results are tabulated from these clinical studies:

	KRA
Positive Agreement	97.5%
Negative Agreement	97.5%
Overall Agreement	97.5%

Analyte	KRA	
	Pos	Neg
Negative Samples	0	20
Near Cut-off Negative Sample (Between 50% of cut-off and cut-off)	1	19
Near Cut-off Positive Samples (Between cut-off and 150% of cut-off)	19	1
Positive Samples (>150% of cut-off)	20	0
Agreement with GC/MS	97.5%	97.5%

#### Reproducibility

Reproducibility studies were carried out using commercially available stock solutions of Kratom analytes. Dilutions were made from the stock solution of Kratom to the concentrations specified in the following table. The results are listed in the following table.

#### KRATOM (KRA)

Kratom Conc. (ng/mL)	Total Number of Determinations	Results	Precision
No drug present	60	60 Negative	>99%
50	60	60 Negative	>99%
150	60	60 Positive	>99%

#### Analytical Sensitivity

A drug-free urine pool was spiked with Kratom at concentration listed. The results are summarized below.

Drug Concentration Cut-off Range	N	KRA	
		-	+
0% Cut-off	30	30	0
-50% Cut-off	30	30	0
-25% Cut-off	30	30	0
Cut-off	30	3	27
+25% Cut-off	30	0	30
+50% Cut-off	30	0	30

#### Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that were detected positive in urine by the **One Step Drug of Abuse Test** at a read time of 5 minutes.

KRATOM (KRA)	Result
(Mitragynine, Cut-off = 100ng/mL)	Positive at 100ng/mL
7-Hydroxymitragynine	Negative at 10,000ng/mL

## EFFECT OF URINARY SPECIFIC GRAVITY

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.005, 1.015, 1.030) were spiked with Kratom drug at 50% below and 50% above cut-off levels respectively. The **One Step Drug of Abuse Test** was tested in duplicate using ten drug-free urine and spiked urine samples. The results demonstrated that varying ranges of urinary specific gravity do not affect the test results.

## EFFECT OF THE URINARY PH

The pH of an aliquoted negative urine pool was adjusted to pH ranges of 4.0, 4.5, 5.0, 6.0 and 9.0, and spiked with drugs at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with the **One Step Drug of Abuse Test**. The results demonstrated that varying ranges of pH do not interfere with the performance of the test.

## INTERFERENCE

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or drug-positive urine containing Kratom. The following compounds show no cross-reactivity when tested with the One Step Drug of Abuse Test at concentrations at 100µg/mL.

### Kratom Non-Cross-Reacting Compounds:

Acebutolol HCl	Ecgonine HCl	D-Norpropoxyphene Maleate Salt
Acepromazine-D6 HCl	Efavirenz	Noroxymorphone HCl
Acetaminophen	Ethylone	Norfentanyl
N-Acetylprocainamide	Ethylmorphine	Noscapine
Acetophenetidin	Fenoprofen	Nylidrin HCl
Alprazolam	Fentanyl	(±)-Octopamine HCl
Alphenal	Furosemide	Oxalic Acid
Amoxicillin	Gentisic Acid	Oxolinic Acid
Ampicillin	D-Glucuronic Acid	Oxycodone
Amitriptyline HCl	Glutethimide	Oxymetazoline
S(+)-Amphetamine	Guaifenesin	Papaverine
R(-)-Amphetamine	Hemoglobin Porcine	PCP
Amobarbital	Heroin HCl	Pentobarbital
(±)-Amphetamine	Hippuric Acid	Pentazocine
R(-)-Apomorphine HCl	Hydralazine HCl	Perphenazine
Aprobarbital	Hydromorphone	Penicillin G Sodium
Aspirin	Hydrocodone	Phenelzine Sulfate Salt
Aspartame	α-Hydroxyhippuric Acid	Phenobarbital
L-Ascorbic Acid	21-Hydroxyprogesterone	Phentermine HCl
Atropine	p-Hydroxymethamphetamine	Phenethylamine
6-Acetylmorphine	Hydrocortisone	L-Phenylephrine
Acetylsalicylic Acid	Hydrochlorothiazide	Phenylpropanolamine HCl
Benzphetamine	4-Hydroamphetamine HCl	Prednisolone
Benzilic Acid	Ibuprofen	Prednisone Acetate
Benzoyllecgonine	Imipramine	Procaine HCl
SS Benzoic Acid	Ipiazid	Promazine HCl
Bilirubin	Isoxsuprine HCl	Promethazine
Brompheniramine Maleate	Isoproterenol HCl	D-Propoxyphene
Buprenorphine	Ketamine HCl	Propranolol HCl
Buspirone HCl	Ketoprofen	Pseudoephedrine
Butalbital	Emetine Dihydrochloride Hydrate	Phenytoin
Butabarbital	(±)-Ephedrine HCl	Quinine
Cannabidiol	[1R,2S]-(-)-Ephedrine	Quinidine
Cannabiol	Erythromycin	Quinacrine
Caffeine	Eserine	Ranitidine HCl
Cetirizine HCl	Estazolam	Sertraline HCl
Chlordiazepoxide HCl	β-Estradiol	Sulfamethazine
Chlorothiazide	(±)-EDDP	Sulindac
Chloroquine	Ethyl-p-aminobenzoate	Temazepam
Chlorpheniramine Maleate	JWH-018 Pentanoic Acid	Terfenadine
Chlorpromazine HCl	JWH-073 Butanoic Acid	Terbutaline
Chloramphenicol	Labetalol HCl	Tetraethylthiuram Disulfide
Chloralhydrate	Levorphanol	Δ-8-THC
Cholesterol	Loperamide HCl	Tetracycline
Chlorothiazide	Lorazepam	Tetrahydrocortisone 3-(β-D-Glucuronide-(-)-Δ9-THC)
Clomipramine	Maprotiline HCl	(±)-11-Hydroxy-Δ-9-THC
Clonazepam	(±)-MDEA	(-)-11-Nor-9-Carboxy-Δ <sup>9</sup> -THC
Clonidine HCl	(±)-MDA	Thebaine
Clozapine	Meperidine	Theophylline
(-)-Cotinine	Meprobamate	Thioridazine
Cocaehtylene	Methamphetamine HCl	Thiamine HCl
Cocaine HCl	(±)-Methadone	DL-Thyroxine
Codeine	S(+)-D-Methamphetamine	Tolbutamide
Cortisone	L-Methamphetamine	Tramadol
Creatinine	Methylphenidate	Triamterene
Cyclopentobarbital	(±)-MDMA	Tryptamine
Citalopram Hydrobromide	(±)-MDPV	Trifluoperazine HCl
Dextromethorphan	Methyprylon	DL-Tryptophan
Desipramine	Morphine	Triazolam
Diazepam	Morphine-3-β-D-Glucuronide	Trans-2-phenylcyclopropylamine HCl
Diclofenac Sodium Salt	Morphine Sulfate Salt	DL-Tyrosine
Dicyclomine	Nalidixic Acid	Tyramine
Digoxin	Nalorphine HCl	Uric Acid
4-Dimethylaminoantipyrine	Nicotinamide	Verapamil HCl
Dihydrocodeine HCl	Nimesulide	Valproic Acid
5,5-Diphenylhydantoin	Nifedipine	Zomepirac
Diphenhydramine	Norcodeine	
Dopamine	Nordoxepin HCl	
Doxylamine	Norfloracin	
Ecgonine Methyl Ester	Norethisterone	

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