Kratom (KRA) Rapid Test
Package Insert

For professional *in vitro* diagnostic use only.

INTENDED USE & SUMMARY

Kratom (KRA) Rapid Test is a lateral flow chromatographic immunoassay for the detection of detection of Mitragynine in human urine at a cut-off concentration of 500 ng/mL. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.
This assay 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 judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Mitragyna speciosa is a tropical evergreen tree in thecoffee family native to Southeast Asia. M. speciosaisindigenous to Thailand, Indonesia, Malaysia, Myanmar, and Papua New Guinea, where it has beenusedin traditional medicines since at least thenineteenth century. Kratom has opioid properties andsome stimulant-like effects.Mitragynine is classified as a kappa-opioid receptor agonist and is roughly 13 times more potent thanmorphine. Mitragynine is thought to be responsible for the opioid-like effectsKratom, due to its opioid-like action, has been used for treatment of pain and opioid withdrawal. Animalstudies suggest that the primary mitragynine pharmacologic action occurs at the mu and delta-opioidreceptors, as well as serotonergic and noradrenergic pathways in the spinal cord. Stimulation atpost-synaptic alpha-2 adrenergic receptors, and receptor blocking at 5-hydroxytryptamine 2A may alsooccur. The 7-hydroxymitragynine may have a higher affinity for the opioid receptors. Partial agonistactivity may be involved.
The Kratom (KRA) Rapid Test is a rapid urine screening test that can be performed without the use of an instrument. The test utilizes a monoclonal antibody to selectively detect elevated levels of Mitragynine in urine. The Kratom (KRA) Rapid Test yields a positive result when the concentration of Mitragynine in urine exceeds 500 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).

PRINCIPLE

The Kratom (KRA) Rapid Test is a rapid chromatographic immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.
During testing, a urine specimen migrates upward by capillary action. Mitragynine, if present in the urine specimen below 500 ng/mL, will not saturate the binding sites of the antibody coated particles in the Test strip. The antibody coated particles will then be captured by immobilized Mitragynine conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Mitragynine level exceeds 500 ng/mL because it will saturate all the binding sites of anti-Mitragynine antibodies.
A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. 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.

COMPOSITION

Each test kit contains test strip/device (dropper)/panel and package insert.
Materials required but not provided: timer, specimen collection container.

STORAGE AND STABILITY

- Store the test kit in a cool, dry place between 2-30°C. Keep away from light. Exposure to temperature and/or humidity outside the specified conditions may cause inaccurate results.
- **Do not freeze.** Use the test kit at temperatures between 15-30°C.
- Use the test kit between 10-90% humidity.
- Do not use the test kit beyond the expiration date (printed on the foil pouch and box).

Note: *All expiration dates are printed in Year-Month-Day format. 2022-06-18 indicates June 18, 2022.*

WARNINGS, PRECAUTIONS AND LIMITATIONS

- For professional in vitro diagnostic use only. Do not use the test after the expiration date.
- The test should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal regulations.
- The Kratom (KRA) Rapid 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.
- It is possible 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.
- A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
- 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.

SPECIMEN COLLECTION AND PREPARATION

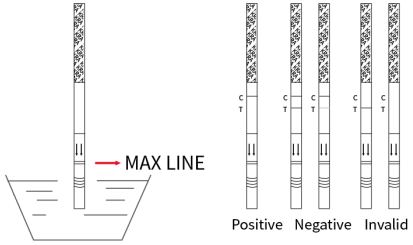
1) Urine Assay
The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear supernatant for testing.

2) Specimen Storage
Urine specimens may be stored at 2-8°C for up to 48 hours prior to assay. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

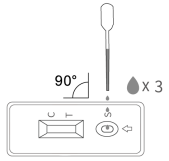
TEST PROCEDURE

Allow the test, urine specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

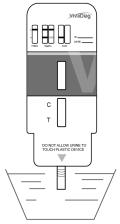
[For Strip]
1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
2. With arrows pointing towards the urine specimen, immerse the test strip vertically in the urine specimen in such a way that urine does not cross MAX line on the test strip for 10-15 seconds. See the illustration below.
3. Wait for the red line(s) to appear. The result should be **read at 5 minutes**. Do not interpret the result after 10 minutes.



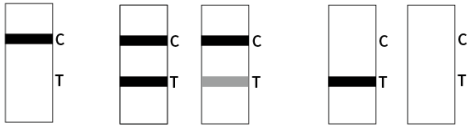
[For Device]
1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx.. 120 µL) to the specimen well (S) of the test device, and then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
3. Wait for the red line(s) to appear. The result should be **read at 5 minutes**. Do not interpret the result after 10 minutes.



[For Panel]
1. Bring the pouch to room temperature before opening it. Remove the test panel from the sealed pouch and use it as soon as possible.
2. With the arrow pointing toward the urine specimen, immerse the test card vertically in the urine specimen for at least 10 to 15 seconds. Immerse the strip to at least the level of the wavy lines, but not above the arrow on the test card.
3. Replace the cap and place the test card on a non-absorbent flat surface.
4. Wait for the red line(s) to appear. The result should be **read at 5 minutes**. Do not interpret the result after 10 minutes.



INTERPRETATION OF TEST RESULTS



Positive: A colored line in the control line region (C) but no line in the test line region (T) for a specific drug indicates a positive result. This indicates that the drug concentration in the specimen exceeds the designated cut-off for that specific drug.

Negative: Two distinct colored lines appear. A colored line in the control line region (C) and a colored line in the test line region (T) for a specific drug indicate a negative result. This indicates that the drug concentration in the specimen is below the designated cut-off level for that specific drug.

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

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test panel. If the problem persists, discontinue using the lot immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is the internal procedural control. This procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

PERFORMANCE

1. Accuracy

A comparison was conducted using the Kratom (KRA) Rapid Test and GC-MS. Testing was performed on 300 clinical specimens previously collected from subjects present for Drug Screen Testing. Ten percent of the specimens employed were either at -25% or +25% level of the cut-off concentration of 500 ng/mL Mitragynine. Presumptive positive results were confirmed by GC/MS. The following results were tabulated:

Method		GC/MS		Total Results
Kratom (KRA) Rapid Test	Results	Positive	Negative	
	Positive	140	9	149
	Negative	3	148	151
Total Results		143	157	300
Positive Agreement		98%		
Negative Agreement		94%		
Total Results		96%		

2. Analytical Sensitivity

A drug-free urine pool was spiked with drugs to the concentrations at ±50% cut-off and ±25% cut-off. The results are summarized below.

A drug-free urine pool was spiked with Mitragynine at the following concentrations: 0 ng/mL, 250 ng/mL, 375 ng/mL, 500 ng/mL, 625 ng/mL and 750 ng/mL. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Mitragynine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
250	-50%	30	30	0
375	-25%	30	25	5
500	Cut-off	30	17	13
625	+25%	30	7	23
750	+50%	30	0	30

3. The following tables list the concentrations of compounds (ng/mL) above which the Kratom (KRA) Rapid Test identified positive results at 5 minutes.

KRA related Compound	Concentration (ng/mL)
Mitragynine	500
7-hydroxymitragynine	1000

4. Precision
Three study sites are participating in the study. Tests were performed over a 10-day period by three operators at each site. There were two tests per day per concentration at each site for each lot to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing, according to GC/MS, no Mitragynine, 25% Mitragynine above and below the cut-off, and 50% Mitragynine above and below the 500 ng/mL cut-off was provided to each site. The results are given below:

Mitragynine Concentration (ng/mL)	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	20	60	0	60	0	60	0
250	20	60	0	60	0	60	0
375	20	48	12	56	4	54	6
625	20	10	50	14	46	16	44
750	20	0	60	0	60	0	60

5. Effect of Specific Gravity

Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 250 ng/mL and 750 ng/mL of Mitragynine. Kratom (KRA) Rapid Test was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

6. Effect of Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 4 to 9 in 1 pH unit increments and spiked with Mitragynine to 250 ng/mL and 750 ng/mL. The spiked, pH-adjusted urine was tested with the Kratom (KRA) Rapid Test in duplicate. The results demonstrate that varying ranges of pH does not interfere with the performance of the test.

INTERFERENCE

A study was conducted to determine the interference of the test with compounds in either drug-free urine or Mitragynine positive urine. The following compounds show no interference when tested with the Kratom (KRA) Rapid Test at a concentration of 100 µg/mL.

NON INTERFERENCE

Acetophenetidin	Cortisone	Isoxsuprine	d-Pseudoephedrine
N-Acetylprocainamide	l-Cotinine	Ketoprofen	Quinidine
Acetylsalicylic acid	Creatinine	Labetalol	Quinine
Aminopyrine	Deoxycorticosterone	Loperamide	Salicylic acid
Amoxicillin	Dextromethorphan	Meprobamate	Serotonin
Ampicillin	Diclofenac	Methoxyphenamine	Sulfamethazine
l-Ascorbic acid	Diflunisal	Methylphenidate	Sulindac
Apomorphine	Digoxin	Nalidixic acid	Tetracycline
Aspartame	Diphenhydramine	Naproxen	Tetrahydrocortisone,
Atropine	Ethyl-p-aminobenzoate	Niacinamide	3-Acetate
Benzilic acid	β-Estradiol	Nifedipine	Tetrahydrocortisone
Benzoic acid	Estrone-3-sulfate	Norethindrone	Tetrahydrozoline
Bilirubin	Erythromycin	Noscapine	Thiamine
d,l-Brompheniramine	Fenoprofen	d,l-Octopamine	Thioridazine
Caffeine	Furosemide	Oxalic acid	d,l-Tyrosine
Cannabidiol	Gentisic acid	Oxolinic acid	Tolbutamide
Chloral hydrate	Hemoglobin	Oxymetazoline	Triamterene
Chloramphenicol	Hydralazine	Papaverine	Trifluoperazine
Chlorothiazide	Hydrochlorothiazide	Penicillin-G	Trimethoprim
d,l-Chlorpheniramine	Hydrocortisone	Perphenazine	d,l-Tryptophan
Chlorpromazine	o-Hydroxyhippuric acid	Phenelzine	Uric acid
Cholesterol	3-Hydroxytyramine	Prednisone	Verapamil
Clonidine	d,l-Isoproterenol	d,l-Propanolol	Zomepirac

REFERENCES

1. Tietz NW. Textbook of Clinical Chemistry. W.B. Saunders Company. 1986; 1735
2. Baselt RC. Disposition of Toxic Multi-Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488
3. Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

INDEX OF SYMBOLS

	Consult instructions for use		Use by		Contains sufficient for <n> tests
IVD	For *in vitro* diagnostic use only	LOT	Lot number	REF	Catalog number
2°C-30°C	Storage temperature limitations		Manufacturer		Do not reuse
EC REP	Authorized Representative				

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CE
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