

# CLIA WAIVED Integrated E-Z Split Key® Cup II

## Instruction Sheet for testing of any combination of the following drugs: AMP/COC/THC/mAMP/MDMA/OPI/PCP

**A rapid, screening test for the simultaneous, qualitative detection of multiple drugs and drug metabolites in human urine.**

**For healthcare professionals including professionals at point of care sites.**

**For in vitro diagnostic use only.**

### INTENDED USE

The Integrated E-Z Split Key® Cup II is a lateral flow immunoassay for the qualitative detection of multiple drugs and drug metabolites in urine at the following cut-off concentrations:

Test	Target Drug	Cut-off
Amphetamine (AMP)	d-Amphetamine	1,000 ng/mL
Cocaine (COC)	Benzoyllecgonine	300 ng/mL
Marijuana (THC)	11-nor- $\Delta^2$ -THC-9-COOH	50 ng/mL
Methamphetamine (mAMP)	d-Methamphetamine	1,000 ng/mL
Methylenedioxyamphetamine (MDMA) Ecstasy	d,l-Methylenedioxyamphetamine	500 ng/mL
Opiates (OPI 2000)	Morphine	2,000 ng/mL
Phencyclidine (PCP)	Phencyclidine	25 ng/mL

**Configurations of the Integrated E-Z Split Key® Cup II can consist of any combination of the drug analytes listed above. 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 indicated.**

### SUMMARY

The Integrated E-Z Split Key® Cup II is a rapid urine screening test that can be performed without the use of an instrument. The test utilizes monoclonal antibodies to selectively detect elevated levels of specific drugs in urine.

#### AMPHETAMINE (AMP)

Amphetamine is a Schedule II controlled substance available by prescription (Dexedrine®) and is also available on the illicit market. Amphetamines are a class of potent sympathomimetic agents with therapeutic applications. They are chemically related to the human body's natural catecholamines: epinephrine and norepinephrine. Acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, reduced appetite, and a sense of increased energy and power. Cardiovascular responses to Amphetamines include increased blood pressure and cardiac arrhythmias. More acute responses produce anxiety, paranoia, hallucinations, and psychotic behavior. The effects of Amphetamines generally last 2-4 hours following use and the drug has a half-life of 4-24 hours in the body. About 30% of Amphetamines are excreted in the urine in unchanged form, with the remainder as hydroxylated and deaminated derivatives.

The Integrated E-Z Split Key® Cup II yields a positive result when Amphetamines in urine exceed 1,000 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).<sup>4</sup>

#### COCAINE (COC)

Cocaine is a potent central nervous system (CNS) stimulant and a local anesthetic. Initially, it brings about extreme energy and restlessness while gradually resulting in tremors, over-sensitivity and spasms. In large amounts, cocaine causes fever, unresponsiveness, difficulty in breathing and unconsciousness. Cocaine is often self-administered by nasal inhalation, intravenous injection and free-base smoking. It is excreted in the urine in a short time primarily as Benzoyllecgonine <sup>2,3</sup>. Benzoyllecgonine, a major metabolite of cocaine, has a longer biological half-life (5-8 hours) than cocaine (0.5-1.5 hours), and can generally be detected for 24-48 hours after cocaine exposure.<sup>3</sup>

The Integrated E-Z Split Key® Cup II yields a positive result when the cocaine metabolite in urine exceeds 300 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).<sup>4</sup>

#### MARIJUANA (THC)

THC ( $\Delta^9$ -tetrahydrocannabinol) is the primary active ingredient in cannabis (marijuana). When smoked or orally administered, THC produces euphoric effects. Users have impaired short term memory and slowed learning. They may also experience transient episodes of confusion and anxiety. Long-term, relatively heavy use may be associated with behavioral disorders. The peak effect of marijuana administered by smoking occurs in 20-30 minutes and the duration is 90-120 minutes after one cigarette. Elevated levels of urinary metabolites are found within hours of exposure and remain detectable for 3-10 days after smoking. The main metabolite excreted in the urine is 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid ( $\Delta^9$ -THC-COOH).

The Integrated E-Z Split Key® Cup II yields a positive result when the concentration of THC-COOH in urine exceeds 50 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).<sup>4</sup>

#### METHAMPHETAMINE (mAMP)

Methamphetamine is an addictive stimulant drug that strongly activates certain systems in the brain. Methamphetamine is closely related chemically to amphetamine, but the central nervous system effects of Methamphetamine are greater. Methamphetamine is made in illegal laboratories and has a high potential for abuse and dependence. The drug can be taken orally, injected, or inhaled. Acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, reduced appetite, and a sense of increased energy and power. Cardiovascular responses to Methamphetamine include increased blood pressure and cardiac arrhythmias. More acute responses produce anxiety, paranoia, hallucinations, psychotic behavior, and eventually, depression and exhaustion. The effects of Methamphetamine generally last 2-4 hours and the drug have a half-life of 9-24 hours in the body. Methamphetamine is excreted in the urine as amphetamine and oxidized and deaminated derivatives. However, 10-20% of Methamphetamine is excreted unchanged. Thus, the presence of the parent compound in the urine indicates Methamphetamine use. Methamphetamine is generally detectable in the urine for 3-5 days, depending on urine pH level.

The Integrated E-Z Split Key® Cup II yields a positive result when the Methamphetamine in urine exceeds 1,000 ng/mL.

#### METHYLENEDIOXYMETHAMPHETAMINE (MDMA)

Methylenedioxyamphetamine (ecstasy) is a designer drug first synthesized in 1914 by a German drug company for the treatment of obesity.<sup>8</sup> Those who take the drug frequently report adverse effects, such as increased muscle tension and sweating. MDMA is not clearly a stimulant, although it has, in common with amphetamine drugs, a capacity to increase blood pressure and heart rate. MDMA does produce some perceptual changes in the form of increased sensitivity to light, difficulty in focusing, and blurred vision in some users. Its mechanism of action is thought to be via release of the neurotransmitter serotonin. MDMA may also release dopamine, although the general opinion is that this is a secondary effect of the drug (Nichols and Oberlander, 1990). The most pervasive effect of MDMA, occurring in virtually all people who took a reasonable dose of the drug, was to produce a clenching of the jaws. The Integrated E-Z Split Key® Cup II yields a positive result when the Methylenedioxyamphetamine in urine exceeds 500 ng/mL.

#### OPIATES (OPI)

Opiate refers to any drug that is derived from the opium poppy, including the natural products, morphine and codeine, and the semi-synthetic drugs such as heroin. Opioid is more general, referring to any drug that acts on the opioid receptor.

Opioid analgesics comprise a large group of substances which control pain by depressing the central nervous system. Large doses of morphine can produce higher tolerance levels, physiological dependency in users, and may lead to substance abuse. Morphine is excreted unmetabolized, and is also the major metabolic product of codeine and heroin. Morphine is detectable in the urine for several days after an opiate dose.<sup>1</sup>

The Integrated E-Z Split Key® Cup II yields a positive result when the morphine in urine exceeds 2,000 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).<sup>4</sup>

#### PHENCYCLIDINE (PCP)

Phencyclidine, also known as PCP or Angel Dust, is a hallucinogen that was first marketed as a surgical anesthetic in the 1950's. It was removed from the market because patients receiving it became delirious and experienced hallucinations.

Phencyclidine is used in powder, capsule, and tablet form. The powder is either snorted or smoked after mixing it with marijuana or vegetable matter. Phencyclidine is most commonly administered by inhalation but can be used intravenously, intra-nasally, and orally. After low doses, the user thinks and acts swiftly and experiences mood swings from euphoria to depression. Self-injurious behavior is one of the devastating effects of Phencyclidine.

PCP can be found in urine within 4 to 6 hours after use and will remain in urine for 7 to 14 days, depending on factors such as metabolic rate, user's age, weight, activity, and diet.<sup>5</sup> Phencyclidine is excreted in the urine as an unchanged drug (4% to 19%) and conjugated metabolites (25% to 30%).<sup>6</sup> The Integrated E-Z Split Key® Cup II yields a positive result when the phencyclidine level in urine exceeds 25 ng/mL. This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).<sup>4</sup>

### PRINCIPLE

The Integrated E-Z Split Key® Cup II is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against their 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 strip. 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 strip 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 strip 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 Amphetamine, Cocaine, Methamphetamine, Methylenedioxyamphetamine, Morphine, THC and Phencyclidine.

### PRECAUTIONS

- For healthcare professionals including professionals at point of care sites.
- For *in vitro* diagnostic use only. Do not use after the expiration date.
- The test cup should remain in the sealed pouch until use.
- The used test cup should be discarded according to federal, state and local regulations.

### STORAGE AND STABILITY

The Integrated E-Z Split Key® Cup II can be stored at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test cups must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

### SPECIMEN COLLECTION AND PREPARATION

#### Urine Specimen

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used.

#### Specimen Storage

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

### MATERIALS

#### Materials Provided

- Integrated E-Z Split Key® Cup II
- Key
- Package insert

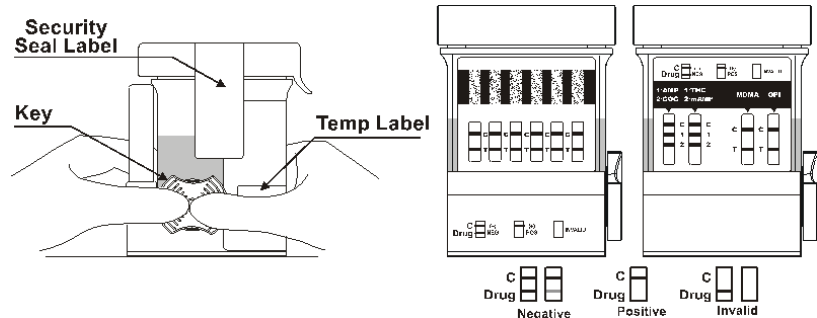
#### Materials Required But Not Provided

- Timer
- External positive and negative controls  
(Please contact the manufacturer for a list of suggested external control suppliers.)

### DIRECTIONS FOR USE

**Allow the test cup, urine sample, and/or controls to reach room temperature (15-30°C) before testing.**

- Bring the pouch to room temperature before opening it. Remove the cup from the sealed pouch and use it as soon as possible.
- Remove the key by twisting it from the center of the cup cap.
- Collect specimen in the cup** and secure cap tightly by pressing down on the pull tab until an audible click is heard.
- Technician dates and initials the security seal and attaches the security seal over the cup cap.
- Place the cup on a flat surface and **push the key to a fully closed position** to initiate the test. Start the timer.
- Remove the peel off label covering the test results.
- Read results at 5 minutes.** The results remain stable for up to sixty minutes.



### INTERPRETATION OF RESULTS

(Please refer to the illustration above)

**NEGATIVE:\* Two lines appear.** One colored line should be in the Control region (C), and another colored line appears in the Test region (T). This negative result indicates that the drugs tested were not present in the urine sample or the drug concentration is below the detectable level of the test.

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

**POSITIVE: One colored 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: No line appears in the Control region (C).** If this occurs, read the directions again and repeat the test with a new panel. If the result is still invalid, stop using the test kit and contact the manufacturer.

## QUALITY CONTROL

A procedural control is included in the test. A colored 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.

It is recommended that external positive and negative controls be tested with each new opened kit, new lot or shipment of product, with each change in operator within the test kit and weekly as a check on continued storage conditions, and as otherwise required by your laboratory's internal quality system procedures. Control specimens should be performed the same as patient specimens (refer to Directions for Use and Interpretation of Results). If unexpected results are seen when running the external positive or negative controls, review the Directions for Use, Interpretation of Results and Limitations sections and repeat the test with another cup. If the problem persists, discontinue use of the test kit immediately and contact the manufacturer (1-800-825-9957).

## LIMITATIONS

- The Integrated E-Z Split Key<sup>®</sup> Cup II 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>3,4,7</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.
- A positive result 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.
- This test does not distinguish between drugs of abuse and certain medications.
- Positive test results may be obtained from certain foods or food supplements.

## PERFORMANCE CHARACTERISTICS

### Accuracy

A side-by-side comparison was conducted using the Integrated E-Z Split Key<sup>®</sup> Cup II and commercially available drug rapid tests. Testing was performed on approximately 300 specimens per drug type previously collected from subjects presenting for drug screen testing. Presumptive positive results were confirmed by GC/MS. The following compounds were quantified by GC/MS and contributed to the total amount of drugs found in presumptive positive urine samples tested.

Test	Compounds Contributing to GC/MS Totals
AMP	Amphetamine
COC	Benzoyllecgonine
THC	11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid
mAMP	Methamphetamine
MDMA	d,l-Methylenedioxyamphetamine
OPI	Morphine, Codeine
PCP	Phencyclidine

The following results are tabulated from these clinical studies:

### %Agreement with GC/MS

	AMP	COC	THC	mAMP	MDMA	OPI	PCP
Positive Agreement	97%	96%	97%	99%	>99%	>99%	>99%
Negative Agreement	95%	90%	88%	94%	98%	90%	97%
Total Results	96%	93%	91%	96%	99%	95%	98%

### %Agreement with Commercial Kit

	AMP	COC	THC	mAMP	MDMA	OPI	PCP
Positive Agreement	97%	95%	98%	98%	>99%	>99%	98%
Negative Agreement	>99%	>99%	>99%	>99%	99%	>99%	>99%
Total Results	98%	98%	99%	99%	99%	>99%	99%

Forty (40) clinical samples for each drug were run using the Integrated E-Z Split Key<sup>®</sup> Cup II by an untrained operator at a Professional Point of Care site. Based on GC/MS data, the operator obtained statistically similar positive agreement, negative agreement and overall agreement rates as trained laboratory personnel.

### Precision

A study was conducted at 3 physician's offices by untrained operators using 3 different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing drug free urine, drug spiked at 25% above and below the cutoff, and drug spiked at 50% above and below the assay cutoff were provided to each site. The correlation with expected results was 98% across all lots and sites with the drug free urine and specimens spiked to +/-50% cutoff for each drug tested (with a 95% confidence interval).

### Analytical Sensitivity

A drug-free urine pool was spiked with drugs to various concentrations. >99% agreement with expected results was found at +/- 50% cutoff for each drug tested (with a 95% confidence interval).

## Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that are detected positive in urine by the Integrated E-Z Split Key<sup>®</sup> Cup II at 5 minutes.

Compound	ng/mL
<b>AMPHETAMINE 1,000</b>	
d-Amphetamine	1,000
d,l-Amphetamine	3,000
l-Amphetamine	50,000
3,4-Methylenedioxyamphetamine (MDA)	2,000
Phentermine	3,000
<b>COCAINE 300</b>	
Benzoyllecgonine	300
Cocaine	780
Cocaethylene	12,500
Ecgonine	32,000
<b>MARIJUANA (THC) 50</b>	
11-nor- $\Delta^9$ -THC-9-COOH	50
Cannabinol	20,000
11-nor- $\Delta^9$ -THC-9-COOH	30
$\Delta^8$ -THC	15,000
$\Delta^9$ -THC	15,000
<b>METHAMPHETAMINE 1,000</b>	
d-Methamphetamine	1,000
p-Hydroxymethamphetamine	30,000
l-Methamphetamine	8,000
3,4-Methylenedioxyamphetamine (MDMA)	2,000
Mephentermine	50,000

Compound	ng/mL
<b>METHYLENEDIOXYMETHAMPHETAMINE (MDMA) 500</b>	
3,4-Methylenedioxyamphetamine (MDMA)	500
3,4-Methylenedioxyamphetamine (MDA)	3,000
3,4-Methylenedioxyethylamphetamine (MDEA)	300
<b>OPIATE 2,000</b>	
Morphine	2,000
Codeine	2,000
Ethylmorphine	5,000
Hydrocodone	12,500
Hydromorphone	5,000
Levorphanol	75,000
6-Monoacetylmorphine (6-MAM)	5,000
Morphine 3- $\beta$ -D-glucuronide	2,000
Norcodeine	12,500
Normorphine	50,000
Oxycodone	25,000
Oxymorphone	25,000
Procaine	150,000
Thebaine	100,000
<b>PHENCYCLIDINE (PCP) 25</b>	
Phencyclidine	25
4-Hydroxyphencyclidine	12,500

### Effect of Urinary Specific Gravity

Urine samples of normal, high, and low specific gravity ranges (1.000-1.037) were spiked with drugs at 50% below and 50% above the assay cutoff levels. The spiked urine samples were tested with the Integrated E-Z Split Key<sup>®</sup> Cup II. The results demonstrate that this varying range of urinary specific gravity levels does not affect the test results.

### Effect of the Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a range of 5 to 9 in 1 pH unit increments and spiked with drugs at 50% below and 50% above the assay cutoff levels. The spiked, pH-adjusted urine was tested with the Integrated E-Z Split Key<sup>®</sup> Cup II. The results demonstrate that these varying ranges of pH (5-9) do not interfere with the performance of the test.

### Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or drug positive urine containing Amphetamine, Cocaine, Marijuana, Methamphetamine, Methylenedioxyamphetamine, Opiates and Phencyclidine. The following compounds show no cross-reactivity when tested with the Integrated E-Z Split Key<sup>®</sup> Cup II at a concentration of 100  $\mu$ g/mL.

### Non Cross-Reacting Compounds

Acetaminophen	Creatinine	Ketoprofen	d-Pseudoephedrine
Acetophenetidin	Deoxycorticosterone	Labetalol	Quinacrine
N-Acetylprocainamide	Dextromethorphan	Loperamide	Quinine
Acetylsalicylic acid	Diclofenac	Meperidine	Quindine
Aminopyrine	Diffunisal	Meprobamate	Rantidine*
Amoxicillin	Digoxin	Methoxyphenamine	Salicylic acid
Ampicillin	Diphenhydramine	Methylphenidate	Serotonin
l-Ascorbic acid	l- $\Psi$ -Ephedrine	Nalidixic acid	Sulfamethazine
Apomorphine	$\beta$ -Estradiol	Naproxen	Sulindac
Aspartame	Estrone-3-sulfate	Niacinamide	Tetracycline
Atropine	Ethyl-p-aminobenzoate	Nifedipine	Tetrahydrocortisone
Benzilic acid	l (-)-Epinephrine	Norethindrone	3-acetate
Benzoic acid	Erythromycin	Noscapine	Tetrahydrocortisone
Benzphetamine*	Fenoprofen	d,l-Octopamine	3-( $\beta$ -D-glucuronide)
Bilirubin	Furosemide	Oxalic acid	Tetrahydrozoline
d,l-Brompheniramine	Gentisic acid	Oxolinic acid	Thiamine
Caffeine	Hemoglobin	Oxymetazoline	Thioridazine
Cannabidiol	Hydralazine	Papaverine	d,l-Tyrosine
Chloralhydrate	Hydrochlorothiazide	Penicillin-G	Tolbutamide
Chloramphenicol	Hydrocortisone	Pentazocine	Triamterene
Chlorothiazide	o-Hydroxyhippuric acid	Perphenazine	Trifluoperazine
d,l-Chloropheniramine	p-Hydroxytyramine	Phenelzine	Trimethoprim
Chlorpromazine	lbutrofen	Trans-2-phenylcyclopropylamine	Tryptamine
Cholesterol	lproniazid	Prednisolone	d,l-Tryptophan
Clonidine	d,l-Isoproterenol	Prednisone	Uric acid
Cortisone	Isosuprine	d,l-Propranolol	Verapamil
l-Cotinine	Ketamine	Ketoprofen	Zomepirac
Acetaminophen	Creatinine		d-Pseudoephedrine

\*Parent compound only.

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