ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ AB-PINACA Assay

This ARK Diagnostics, Inc. package insert for the ARK AB-PINACA Assay must be read prior to use. Package insert instructions must be followed accordingly. The assay provides a simple and rapid analytical screening procedure for detecting AB-PINACA and its metabolites in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable.

Customer Service

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CE 2797

EC REP

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE 2797	CE Mark with notified body number
	Consult Instructions for Use	R1 R2	Reagent 1 / Reagent 2
	Temperature limitation	IVD	In Vitro Diagnostic Medical Device
Rx Only	For Prescription Use Only		

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Reagent Kit REF 5055-0001-00

Reagent Kit REF 5055-0001-01

Reagent Kit REF 5055-0001-02

1 Name

ARK™ AB-PINACA Assay

2 Intended Use

The ARK AB-PINACA Assay is an immunoassay intended for the qualitative determination of AB-PINACA and its metabolites in human urine at a cutoff concentration of 5 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This *in vitro* diagnostic device is for prescription use only.

The ARK AB-PINACA Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed positive analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

3 Summary and Explanation of Test

Synthetic cannabinoids are part of a group of drugs called new psychoactive substances (NPS), which are designer drugs intended to mimic the effects of illicit drugs. These substances are called cannabinoids because they interact with the same CB_1 and CB_2 cannabinoid receptors as tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana. Although synthetic cannabinoids are functionally similar to THC, many of these substances are not structurally related to THC. Synthetic cannabinoids became popular under the brand names “Spice” and “K2”, in part due to their ability to escape detection by standard cannabinoid screening tests. Synthetic cannabinoids are marketed under a wide variety of specific brand names, including Joker, Black Mamba, Kush, and Kronic. Synthetic cannabinoids are used in a variety of ways, with the most common method being sprayed onto dried plant material and smoked. Potential adverse effects of synthetic cannabinoid use include anxiety, agitation, hallucinations, dizziness, seizures, rapid heart rate and vomiting.[1-9]

4 Principles of the Procedure

The ARK AB-PINACA Assay is a homogeneous enzyme immunoassay method used for the analysis of drug in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 Reagents

REF	Product Description	Quantity/Volume
5055-0001-00	**ARK AB-PINACA Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to AB-PINACA metabolite, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme AB-PINACA derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 14 mL

REF	Product Description	Quantity/Volume
5055-0001-01	**ARK AB-PINACA Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to AB-PINACA metabolite, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme AB-PINACA derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 58 mL

REF	Product Description	Quantity/Volume
5055-0001-02	**ARK AB-PINACA Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to AB-PINACA metabolite, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 58 mL
	Reagent R2 – Enzyme AB-PINACA derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 29 mL

Reagent Handling and Storage

ARK AB-PINACA Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK AB-PINACA products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 Warnings and Precautions

- For *In Vitro* Diagnostic Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Do not use reagents after the expiration date.
- Reagents contain ≤0.09% sodium azide.

7 Specimen Collection and Preparation for Analysis

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- Human urine is required. Treat as potentially infectious material.
- Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical and physical integrity of the urine sample from the time it is collected until the time it is assayed, including during transport. Fresh urine specimens are suggested.
- Cap the urine sample immediately after collection, store refrigerated at 2-8°C (36–46°F) and assay within 7 days after collection. If the assay cannot be performed within 7 days, store the urine sample frozen at -20°C.[10]
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- The presence of bubbles or foam on specimens may lead to short sample delivery and erroneous results.
- Each laboratory should consult available literature and internal data regarding specimen stability. The recommended pH range for urine specimens is 4.0 – 11.0.[11]
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

8 Procedure

Materials Provided

ARK AB-PINACA Assay – REF 5055-0001-00, 5055-0001-01 or 5055-0001-02

Materials Required – Provided Separately

ARK AB-PINACA Negative Calibrator – REF 5055-0002-01

ARK AB-PINACA Cutoff Calibrator – REF 5055-0002-02

Quality Controls – ARK AB-PINACA Control – REF 5055-0003-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Many automated clinical chemistry analyzers with photometric rate determination

at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK AB-PINACA Assay, available from your distributor or ARK

Customer Service. Application Protocol Sheets bearing the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Qualitative Results
Use the 5 ng/mL Cutoff Calibrator to distinguish negative and positive samples. Run the Low and High Controls as Negative and Positive respectively. Report test results less than the response value for the Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the Cutoff Calibrator as Positive.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols
- A stored calibration curve was effective up to at least 29 days based on supporting data

Quality Control (QC) and Calibration
Laboratories should establish QC procedures for the ARK AB-PINACA Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Each laboratory should establish its own ranges for each new lot of controls. Control results should fall within established ranges as determined by laboratory procedures and guidelines. The ARK AB-PINACA Control is intended for use in quality control of the ARK AB-PINACA Assay.

The Low Control should be Negative and the High Control should be Positive relative to the 5 ng/mL Cutoff Calibrator.

9 Results and Expected Values

The actual concentration of drug and its metabolites cannot be determined. A confirmatory method is required.

Qualitative Analysis – Negative Results

A specimen that gives a response value less than the ARK AB-PINACA Cutoff Calibrator response value is interpreted as negative.

Qualitative Analysis – Positive Results
A specimen that gives a response value equal to or greater than the ARK AB-PINACA Cutoff Calibrator response value is interpreted as positive.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

10 Limitations

- The assay is designated for use with human urine only.
- ARK AB-PINACA Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK AB-PINACA Assay indicates only the presence of drug and its metabolites and does not necessarily correlate with the extent of physiological and psychological effects.
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- It is possible that substances other than those tested in the specificity study may interfere with the test and cause false results.

11 Specific Performance Characteristics

The following performance characteristics were collected on the Beckman Coulter AU680® automated clinical chemistry analyzer using the ARK AB-PINACA Assay.

Precision
Drug-free, negative human urine was supplemented with AB-PINACA Pentanoic Acid (calibrator analyte) ranging from 0.0 to 10.0 ng/mL. Each level was assayed in quadruplicate twice a day for 20 days (N=160). Results are summarized in the table below.

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.00	-100	160	160 Negative
1.25	-75	160	160 Negative
2.50	-50	160	160 Negative
3.75	-25	160	160 Negative
5.00	Cutoff	160	67 Negative; 93 Positive
6.25	+25	160	160 Positive
7.50	+50	160	160 Positive
8.75	+75	160	160 Positive
10.00	+100	160	160 Positive

Analytical Specificity

AB-PINACA Metabolites and Structurally Related Compounds

Synthetic cannabinoids are extensively metabolized, with little to no unchanged parent drug found in human urine. The active metabolites of synthetic cannabinoids may prolong the parent drug's psychotropic effects and contribute to its toxicological profile.[12-22]

All compounds tested were added to drug-free, negative human urine.

The cross-reactivity of the following AB-PINACA metabolites and structurally related compounds was evaluated by spiking these compounds into drug-free, negative human urine to determine the minimum concentration that would give a positive result approximately equivalent to the 5 ng/mL cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:

% Cross-reactivity = (Cutoff concentration / Lowest concentration of cross-reactant causing a positive result) X 100

Compound	Concentration (ng/mL)	Percent Cross-reactivity (%)
AB-PINACA	8.0	62.50
AB-PINACA N-(4-hydroxypentyl)	5.0	100.00
AB-PINACA N-(5-hydroxypentyl)	5.0	100.00
5-fluoro AB-PINACA	4.7	106.38
5-fluoro ABICA	6.0	83.33
5-fluoro ADBICA	6.0	83.33
5-fluoro AB PINACA N-(4-hydroxypentyl)	8.0	62.50
5-fluoro ADB-PINACA	4.2	119.05
5-chloro AB-PINACA	8.0	62.50
ADB-PINACA	9.0	55.56
ADB-PINACA pentanoic acid	3.5	142.86
ADB-PINACA N-(4-hydroxypentyl)	4.0	125.00
ADB-PINACA N-(5-hydroxypentyl)	4.3	116.28
AB-FUBINACA	9.0	55.56
ADB-FUBINACA	10.0	50.00
ADBICA	20.0	25.00
ADBICA N-pentanoic acid	7.0	71.43
ADBICA N-(4-hydroxypentyl)	6.0	83.33
ADBICA N-(5-hydroxypentyl)	5.5	90.91
AB-CHMINACA	11.5	43.48
MAB-CHMINACA (ADB-CHMINACA)	8.5	58.82

High concentrations of the following structurally related compounds were added to drug-free, negative human urine and tested with the ARK AB-PINACA Assay. The compounds at the concentrations listed below were negative when tested with the ARK AB-PINACA Assay.

Compound	Concentration Tested (ng/mL)
AM 2201 6-OH indole	100,000
AM 2201 N-(4-OH pentyl)	20,000
AM 2201	20,000
JWH-007	100,000
JWH-015	50,000

Compound	Concentration Tested (ng/mL)
JWH-019	100,000
JWH-022	50,000
JWH-073	40,000
JWH-081	100,000
JWH-122	100,000
JWH-398	100,000
JWH-018 4-OH indole	100,000
JWH-018 5-OH indole	100,000
JWH-073 N-butanoic acid	40,000
JWH-073 6-OH indole	100,000
JWH-073 N-(4-OH butyl)	15,000
3-(1-napthyl)1H-indole	100,000
BB-22	100,000
BB-22 3-carboxyindole	100,000
PB-22	100,000
PB-22 N-(5-OH pentyl)	60,000
PB-22 pentanoic acid	50,000
UR-144-N-heptyl	100,000
JWH 250 5-OH indole	100,000
RCS-4-2 methoxy isomer	20,000
JWH 250 N-(5-carboxypentyl)	10,000
AM-2232	100,000
AM-2233	50,000

Structurally Unrelated Compounds

The following structurally unrelated compounds were added to drug-free, negative human urine and tested with the ARK AB-PINACA Assay. The compounds at the concentrations listed below were negative when tested with the ARK AB-PINACA Assay.

Compound	Concentration Tested (ng/mL)
4-bromo-2,5-dimethoxyphenethylamine	100,000
6-Acetylcodeine	100,000
6-Acetylmorphine	100,000
7-Aminoclonazepam	100,000
7-Aminoflunitrazepam	100,000
7-Aminonitrazepam	100,000
11-nor-9-carboxy-Δ9-THC	100,000
Acetaminophen	500,000
Acetylsalicylic Acid	500,000
Alprazolam	100,000
Amitriptyline	100,000
Amobarbital	100,000
S-(+)-Amphetamine	100,000
Benzylpiperazine	100,000

Compound	Concentration Tested (ng/mL)
Bromazepam	100,000
Buprenorphine	100,000
Bupropion	100,000
Butabarbital	100,000
Butalbital	100,000
Caffeine	500,000
Cannabidiol	100,000
Cannabinol	100,000
Carbamazepine	100,000
Carisoprodol	100,000
Chlordiazepoxide	100,000
Chlorpromazine	100,000
cis-Tramadol	100,000
Clobazam	100,000
Clomipramine	100,000
Clonazepam	100,000
Clozapine	100,000
Codeine	100,000
Cotinine	100,000
Cyclobenzaprine	100,000
Dehydronorketamine	50,000
Desalkylflurazepam	100,000
Demoxepam	100,000
Desipramine	100,000
Dextromethorphan	100,000
Diazepam	100,000
Digoxin	100,000
Dihydrocodeine	100,000
Δ9-THC	100,000
Diphenhydramine	500,000
Doxepin	100,000
EDDP	100,000
EMDP	100,000
1R,2S (-) Ephedrine	100,000
1S,2R (+) Ephedrine	100,000
Ethyl-β-D-Glucuronide	100,000
Ethylmorphine	100,000
Fenfluramine (+)	100,000
Fenfluramine (-)	100,000
Fentanyl	100,000
Flunitrazepam	100,000
Fluoxetine	100,000
Flurazepam	100,000
Haloperidol	100,000
Heroin	100,000
Hexobarbital	100,000
Hydrocodone	100,000
Hydromorphone	100,000
11-hydroxy-Δ9-THC	100,000
Ibuprofen	500,000
Imipramine	100,000
Ketamine	100,000
Lamotrigine	100,000

Compound	Concentration Tested (ng/mL)
Levorphanol Tartrate	100,000
Lidocaine	100,000
Lorazepam	100,000
Lorazepam Glucuronide	50,000
Lormetazepam	100,000
LSD	100,000
Maprotiline	100,000
(+)-MDA	100,000
MDEA	100,000
MDMA	100,000
Meperidine	100,000
Meprobamate	100,000
Methadone	500,000
S(+)-Methamphetamine	100,000
Methaqualone	100,000
Methoxetamine	100,000
Methylone	100,000
Methylphenidate	100,000
Midazolam	100,000
Morphine	100,000
Morphine-3β-D-Glucuronide	50,000
Morphine-6β-D-Glucuronide	50,000
N-Desmethyltapentadol	100,000
Nalorphine	100,000
Naloxone	100,000
Naltrexone	100,000
Naproxen	100,000
Nitrazepam	100,000
Norbuprenorphine	50,000
Norcodeine	100,000
Nordiazepam	100,000
Norketamine	100,000
Normorphine	100,000
Norpropoxyphene	100,000
Norpseudoephedrine	100,000
Nortriptyline	100,000
Olanzapine	100,000
Oxazepam	100,000
Oxycodone	100,000
Oxymorphone	100,000
PCP	100,000
Pentazocine	100,000
Phentermine	100,000
Pentobarbital	100,000
Phenobarbital	100,000
Phenylephrine	100,000
Phenylpropanolamine	100,000
Phenytoin	100,000
PMA	100,000
Prazepam	100,000
Propoxyphene	100,000
Propranolol	100,000
Protriptyline	100,000

Compound	Concentration Tested (ng/mL)
R,R (+)- Pseudoephedrine	100,000
S,S (-)- Pseudoephedrine	100,000
Ranitidine	100,000
Ritalinic Acid	100,000
Salicylic Acid	100,000
Secobarbital	100,000
Sertraline	100,000
Sufentanil Citrate	50,000
Tapentadol	100,000
Temazepam	100,000
Theophylline	100,000
Thioridazine	100,000
Triazolam	100,000
Trifluoromethylphenylpiperazine	100,000
Trimipramine	100,000
Trazodone	100,000
Venlafaxine	100,000
Verapamil	100,000
Zolpidem Tartrate	100,000

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added to urine spiked with AB-PINACA Pentanoic Acid at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK AB-PINACA Assay.

Compound	Concentration Tested	2.5 ng/mL (-50% Cutoff)	7.5 ng/mL (+50% Cutoff)
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin – Conjugated	2 mg/dL	Negative	Positive
Bilirubin – Unconjugated	2 mg/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Positive
Creatinine	500 mg/dL	Negative	Positive
Ethanol	1000 mg/dL	Negative	Positive
Galactose	10 mg/dL	Negative	Positive
Glucose	2000 mg/dL	Negative	Positive
Hemoglobin	300 mg/dL	Negative	Positive
Human Albumin	500 mg/dL	Negative	Positive
Human Gamma Globulin	500 mg/dL	Negative	Positive
Oxalic Acid	100 mg/dL	Negative	Positive
Riboflavin	7.5 mg/dL	Negative	Positive
Sodium Azide	1% w/v	Negative	Positive
Sodium Chloride	6000 mg/dL	Negative	Positive
Sodium Fluoride	1% w/v	Negative	Positive

Compound	Concentration Tested	2.5 ng/mL (-50% Cutoff)	7.5 ng/mL (+50% Cutoff)
Urea	6000 mg/dL	Negative	Positive

Interference – Specific Gravity and pH

Urine samples with specific gravity values ranging from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of AB-PINACA Pentanoic Acid at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK AB-PINACA Assay.

Method Comparison

A total of seventy (70) unaltered clinical human urine specimens that are not individually identifiable were tested with the ARK AB-PINACA Assay in qualitative mode and the results were compared to another commercially available immunoassay screening method as a reference. Results are summarized in the table below.

		Comparative Screening Method	
ARK AB-PINACA Assay (5 ng/mL Cutoff)		(+)	(-)
	(+)	14	2*
	(-)	0	54

*These two (2) samples were confirmed to be positive by LC-MS/MS.

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13 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.
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