ARK Diagnostics, Inc.

For Criminal Justice and Forensic Use Only

ARK™ *Xylazine Assay*

This ARK Diagnostics, Inc. package insert for the ARK Xylazine 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 Xylazine in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.

48089 Fremont Blvd

Fremont, CA 94538 USA

Tel: 1-877-869-2320

Fax: 1-510-270-6298

customersupport@ark-tdm.com

www.ark-tdm.com

KEY TO SYMBOLS USED

LOT	Batch Code	YYYY-MM-DD	Use by/Expiration Date
REF	Catalog Number		Manufacturer
	Consult Instructions for Use	R1 R2	Reagent 1/Reagent 2
	Temperature Limitation		

1 NAME

ARK™ *Xylazine Assay*

2 INTENDED USE

For Criminal Justice and Forensic Use Only

The ARK Xylazine Assay is a homogeneous enzyme immunoassay intended for the qualitative detection and/or semi-quantitative estimation of xylazine and its metabolites in human urine at a cutoff concentration of 10 ng/mL. The assay is intended for use in laboratories with automated chemistry analyzers.

The semi-quantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK Xylazine 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. Confirmatory testing and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

3 SUMMARY AND EXPLANATION OF THE TEST

Xylazine is a veterinary drug that belongs to a class of compounds known as alpha-2 adrenergic agonists. It is a strong CNS depressant primarily used as a sedative, muscle relaxant, and analgesic in veterinary medicine. Xylazine is commonly administered to animals, particularly in the treatment of large animals like horses, cattle, and deer. It is not approved for human use.[1] As a street drug, xylazine is known as “tranq” or “tranq-dope” and has been found more frequently in illegal drugs in the United States, often mixed with opioids like fentanyl, a highly potent synthetic opioid.[2] People who use illicit drugs, such as fentanyl, may not be aware that xylazine is present. It’s typically injected but can also be ingested or snorted. Xylazine intensifies and makes the effects of fentanyl unpredictable, increasing the risk of overdose. The drug combination poses dangers to public health and safety, causing severe injuries, infections, and even amputations.[3-4]

Xylazine is currently not scheduled under the US Controlled Substance Act.

4 PRINCIPLES OF THE PROCEDURE

The ARK Xylazine Assay is a homogeneous enzyme immunoassay. The assay uses specific antibodies that can detect xylazine in human urine. The assay is based on competition between a drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, rabbit polyclonal anti-xylazine antibody binds to the drug labeled with rG6PDH and causes a decrease in enzyme activity. In the presence of xylazine from the specimen, enzyme activity increases and is directly related to the xylazine concentration. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the conversion of nicotinamide adenine dinucleotide (NAD) to NADH.

5 REAGENTS

REF	Product Description	Quantity/Volume
5088-0001-00	**ARK Xylazine Assay** **Reagent R1 – Antibody/Substrate** Rabbit polyclonal antibodies to xylazine, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Xylazine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 14 mL

Reagent Kit REF 5088-0001-00

Reagent Kit REF 5088-0001-01

REF	Product Description	Quantity/Volume
5088-0001-01	**ARK Xylazine Assay** **Reagent R1 – Antibody/Substrate** Rabbit polyclonal antibodies to xylazine, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme Xylazine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 58 mL

Reagent Handling and Storage

ARK Xylazine 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 Xylazine 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

- Not for *In Vitro* Diagnostic Use.
- 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 for up to 2 months prior to analysis[5-6].
- To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing.
- The presence of bubbles or foam on the sample may lead to short sample delivery and erroneously low results.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- The recommended pH range for urine specimens is 4.0 – 11.0[7].
- 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 Xylazine Assay – REF 5088-0001-00, 5088-0001-01

Materials Required – Provided Separately

ARK Xylazine Calibrator (Set) – REF 5088-0002-00
ARK Xylazine Calibrator A (Negative) – REF 5088-0002-01
ARK Xylazine Calibrator B (Cutoff) – REF 5088-0002-02
ARK Xylazine Control (5.0 ng/mL and 15.0 ng/mL) – REF 5088-0003-00

Instruments

Many automated chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Xylazine Assay, available from your distributor or ARK Customer Service. Refer to the instrument-specific operator's manual for daily maintenance.

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

Assay Sequence

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

Qualitative Results

Use the 10 ng/mL Calibrator B as a 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 rate (mA/min) value for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate (mA/min) value for the Cutoff Calibrator as Positive.

Semi-quantitative Results

Perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with the ARK Xylazine Assay Low and High quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK Xylazine calibrator level (500 ng/mL) may be diluted in ARK Xylazine Calibrator A (Negative urine) and retested.

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

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Xylazine 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 Xylazine Control is intended for use in quality control of the ARK Xylazine Assay.

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the 10 ng/mL Cutoff Calibrator.

9 RESULTS AND EXPECTED VALUES

A more specific confirmatory method, such as LC-MS/MS or GC-MS, is required in order to obtain a confirmed positive result.

Qualitative Analysis – Negative Results

A specimen that gives a rate (mA/min) value less than the ARK Xylazine Calibrator B Cutoff rate (mA/min) value is interpreted as negative; either the specimen does not contain Xylazine or Xylazine is present in a concentration below the cutoff level of this assay.

Qualitative Analysis – Positive Results

A specimen that gives a rate (mA/min) value equal to or greater than the ARK Xylazine Calibrator B Cutoff rate (mA/min) value is interpreted as positive, indicating that Xylazine is present.

Semi-quantitative Analysis

The actual Xylazine concentration cannot be determined with this assay. Semi-quantitative results for positive specimens enable the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semi-quantitative results also permit the laboratory to establish quality control procedures and assess reproducibility. Specimens with sample results above the highest ARK Xylazine calibrator level (500 ng/mL) may be diluted in ARK Xylazine Calibrator A (Negative urine) and retested.

10 LIMITATIONS

- The assay is designated for use with human urine only.
- ARK Xylazine Assay reagents, ARK Xylazine calibrators and ARK Xylazine controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Xylazine Assay indicates only the presence of xylazine and does not necessarily correlate with the extent of physiological and psychological effects.
- **Do not use Boric Acid as a preservative.**
- 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 AU480® automated chemistry analyzer using the ARK Xylazine Assay.

Precision

Drug-free, negative human urine was supplemented with Xylazine (0 to 20 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated qualitatively and semi-quantitatively. Results are summarized in the tables below.

Qualitative Precision

Xylazine (ng/mL)	Relative % Cutoff	# of Results	Results
0.0	-100	160	160 Negative
2.5	-75	160	160 Negative
5.0	-50	160	160 Negative
7.5	-25	160	160 Negative
10.0	Cutoff	160	122 Negative / 38 Positive
12.5	+25	160	160 Positive
15.0	+50	160	160 Positive
17.5	+75	160	160 Positive
20.0	+100	160	160 Positive

Semi-quantitative Precision

Xylazine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0.0	-100	160	0.0	160 Negative
2.5	-75	160	1.7	160 Negative
5.0	-50	160	4.2	160 Negative
7.5	-25	160	6.7	160 Negative
10.0	Cutoff	160	9.5	118 Negative / 42 Positive
12.5	+25	160	11.8	1 Negative / 159 Positive
15.0	+50	160	14.4	160 Positive
17.5	+75	160	17.0	160 Positive
20.0	+100	160	20.2	160 Positive

Analytical Recovery

Drug-free, negative human urine was spiked with Xylazine across the assay range of the semi-quantitative calibration curve. Each sample was run in replicates of 5 in semi-quantitative mode and the average was used to determine percent recovery compared to the expected value.

Expected Value (ng/mL)	Observed Value (ng/mL)	Recovery (%)
6.0	5.8	96.3
9.0	9.0	100.0
12.0	11.6	97.0
20.0	20.1	100.6
60.0	55.5	92.5
150.0	134.2	89.4
400.0	351.0	87.7

Analytical Specificity

All compounds tested were added to drug-free, negative human urine and tested with the ARK Xylazine Assay in both qualitative and semi-quantitative modes.

The cross-reactivity of the following metabolites and alpha-2 agonists and antagonists was evaluated by spiking these compounds into drug-free, negative human urine and evaluated by dose-response to determine the approximate equivalence to the 10 ng/mL xylazine cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:

% Cross-reactivity = (Cutoff concentration / Concentration approximately equivalent to the 10 ng/mL cutoff) X 100

For compounds that did not produce a positive result, the highest concentration tested was used to calculate percent cross-reactivity.

Cross-reactivity of Xylazine Metabolites

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
3-Hydroxy xylazine	9.1	109.8
4-Hydroxy xylazine	18.9	53.0
4-Hydroxy xylazine glucuronide	38.2	26.2

Cross-reactivity of Alpha-2 Agonists and Antagonists

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
Clonidine	2,260	0.4
Romifidine	2,780	0.4
Tizanidine	9,350	0.1
Brimonidine	15,190	0.1
Atipamezole	>100,000	<0.01
Detomidine	>100,000	<0.01
Dexmedetomidine	>100,000	<0.01
Etomidate	>100,000	<0.01
Eutylone	>100,000	<0.01
Guanfacine	>100,000	<0.01
Medetomidine	>100,000	<0.01
Metamizole	>100,000	<0.01
Tolazoline	>100,000	<0.01
Yohimbine	>100,000	<0.01

The following opioids were negative at the concentrations tested with the ARK Xylazine Assay.

Opioid Compounds

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
6-Acetyl morphine	100,000	Naloxone	100,000
Buprenorphine	100,000	Naltrexone	100,000
Codeine	150,000	Norbuprenorphine	100,000
Dextromethorphan	100,000	Norfentanyl	100,000
EDDP	100,000	Norcodeine	100,000
EMDP	100,000	Normorphine	100,000
Ethyl morphine	100,000	Noroxycodone	100,000
Fentanyl	100,000	Nortilidine	100,000
Heroin	100,000	Oxycodone	100,000
Levorphanol	100,000	Oxymorphone	100,000
Meperidine	100,000	Pentazocine	100,000
Methadone	100,000	Tapentadol	100,000
Morphine	100,000	Thebaine	100,000
Nalbuphine	100,000	Tilidine	100,000
Naloxegol	100,000	Tramadol	100,000

The following structurally unrelated compounds were negative at the concentrations tested with the ARK Xylazine Assay.

Structurally Unrelated Compounds

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
(+)-MDA	100,000	Imipramine	100,000
11-Hydroxy-delta-9-THC	100,000	Ketamine	100,000
11-Nor-9-carboxy-THC	100,000	Ketorolac	100,000
1R,2S(-)-Ephedrine	100,000	Lamotrigine	100,000
1S,2R(+)-Ephedrine	100,000	Lidocaine	100,000
4-Bromo-2,5-Dimethoxyphenethylamine	100,000	LSD	100,000
7-Aminoclonazepam	100,000	Maprotiline	100,000
Acetaminophen	1000,000	MDMA	100,000
Acetylsalicylic acid	1000,000	Meprobamate	100,000
Alprazolam	100,000	Metformin	100,000
Amitriptyline	100,000	Methylphenidate	100,000
Amobarbital	100,000	Metronidazole	100,000
Amoxicillin	100,000	Mirtazapine	100,000
Amphetamine	100,000	Naproxen	100,000
Atorvastatin	100,000	Norpseudoephedrine	100,000
Benzoylecgonine	100,000	Nortriptyline	100,000
Benzylpiperazine	100,000	Ofloxacin	100,000
Bupropion	100,000	Omeprazole	100,000
Butabarbital	100,000	Ondansetron	100,000
Caffeine	100,000	Oxazepam	100,000
Canagliflozin	100,000	Phencyclidine	100,000
Cannabidiol	100,000	Phenobarbital	100,000
Cannabinol	100,000	Phentermine	100,000
Carbamazepine	100,000	Phenylephrine	100,000
Carisoprodol	100,000	Phenylpropanolamine	100,000
Chlordiazepoxide	100,000	Phenytoin	100,000

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
Chlorpromazine	100,000	PMA	100,000
Cimetidine	100,000	Propranolol	100,000
Ciprofloxacin	100,000	Protriptyline	200,000
Clobazam	100,000	Quetiapine	100,000
Clomipramine	100,000	R,R(-)-Pseudoephedrine	100,000
Clopidogrel	100,000	Ranitidine	100,000
Cocaine	100,000	Ritalinic Acid	100,000
Cotinine	100,000	S(+)-Methamphetamine	100,000
Cyclobenzaprine	100,000	S,S(+)-Pseudoephedrine	100,000
Desipramine	100,000	Salicylic Acid	100,000
Desmethyl Ofloxacin	100,000	Secobarbital	100,000
Diazepam	100,000	Sertraline	100,000
Diphenhydramine	500,000	Temazepam	100,000
Doxepin	100,000	Theophylline	100,000
Ecgonine	100,000	Thioridazine	100,000
Fluoxetine	500,000	Trazodone	100,000
Fluphenazine	100,000	Triazolam	100,000
Gabapentin	100,000	Trimipramine	100,000
Hydroxyzine	100,000	Venlafaxine	100,000
Ibuprofen	1,000,000	Zolpidem	100,000

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into xylazine-spiked urine (± 50% of the cutoff concentration). No interference was observed when tested with the ARK Xylazine Assay.

Compound	Concentration Tested (mg/dL)	5 ng/mL (-50% Cutoff)	15 ng/mL (+50% Cutoff)
Acetone	500	Negative	Positive
Ascorbic acid	150	Negative	Positive
Bilirubin	2	Negative	Positive
Boric Acid	1 % w/v	Negative	Positive
Creatinine	400	Negative	Positive
Ethanol	10	Negative	Positive
Galactose	5	Negative	Positive
Glucose	1000	Negative	Positive
Hemoglobin	150	Negative	Positive
Human Albumin	200	Negative	Positive
Human γ- Globulin	500	Negative	Positive
NaCl	1000	Negative	Positive
Oxalic Acid	50	Negative	Positive
Riboflavin	3	Negative	Positive
Urea	1000	Negative	Positive

Interference – Specific Gravity and pH

Urine samples with specific gravity values 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 xylazine at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK Xylazine Assay.

Method Comparison

One hundred forty-seven (147) unaltered urine specimens that are not individually identifiable were analyzed by ARK Xylazine Assay in both qualitative and semi-quantitative modes and the results were compared to LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory.

Results are summarized as follows:

ARK Xylazine Assay Results	Low Negative (Less than 50% below the cutoff concentration by LC-MS/MS) (<5.0 ng/mL)	Near Cutoff Negative (Between the cutoff and 50% below the cutoff concentration by LC-MS/MS) (5.0-9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (10.0-15.0 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>15.0 ng/mL)
Positive	0	1*	2	30
Negative	109	5	0	0

*One (1) sample was considered discordant due to disagreement between the methods in calling a positive or negative result relative to the 10 ng/mL cutoff. The sample contained 7.4 ng/mL xylazine by LC-MS/MS and 11.8 ng/mL by the ARK Xylazine Assay.

12 REFERENCES

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13 TRADEMARKS

ARK Diagnostics, Inc.
Fremont, CA 94538 USA

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