

ALL TEST **BZO Rapid Test Dipstick (Urine)**
Package Insert
REF DBZ-101/111 English

A rapid test for the qualitative detection of Benzodiazepines in human urine.
 For medical and other professional *in vitro* diagnostic use only.

[INTENDED USE]
 The BZO Rapid Test Dipstick (Urine) is a rapid chromatographic immunoassay for the detection of Oxazepam (major metabolite) in urine at a cut-off concentration of 300ng/ml. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.

This assay provides only a qualitative, 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.

[SUMMARY]
 Benzodiazepines are medications that are frequently prescribed for the symptomatic treatment of anxiety and sleep disorders. They produce their effects via specific receptors involving a neurochemical called gamma aminobutyric acid (GABA). Because they are safer and more effective, Benzodiazepines have replaced Barbiturates in the treatment of both anxiety and insomnia. Benzodiazepines are also used as sedatives before some surgical and medical procedures, and for the treatment of seizure disorders and alcohol withdrawal.

Risk of physical dependence increases if Benzodiazepines are taken regularly (e.g., daily) for more than a few months, especially at higher than normal doses. Stopping abruptly can bring on such symptoms as trouble sleeping, gastrointestinal upset, feeling unwell, loss of appetite, sweating, trembling, weakness, anxiety and changes in perception. Only trace amounts (less than 1%) of most Benzodiazepines are excreted unaltered in the urine; most of the concentration in urine is conjugated drug. The detection period for the Benzodiazepines in the urine is 3-7 days.

The BZO Rapid Test Dipstick (Urine) is a rapid urine-screening test that can be performed without the use of an instrument. The test utilizes the antibody to selectively detect elevated levels of Benzodiazepines in urine. The BZO Benzodiazepines Test Dipstick (Urine) yields a positive result when the Benzodiazepines in urine exceeds the cut-off level.

[PRINCIPLE]
 The BZO Rapid Test Dipstick (Urine) is an 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. Benzodiazepines, if present in the urine specimen below the cut-off level, will not saturate the binding sites of the antibody in the test. The antibody coated particles will then be captured by immobilized Benzodiazepines-protein 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 Benzodiazepines level exceeds the cut-off level, because it will saturate all the binding sites of anti-Benzodiazepines antibody.

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.

[REAGENTS]
 The test contains mouse monoclonal anti-Benzodiazepines antibody coupled particles and Benzodiazepines-protein conjugate. A goat antibody is employed in the control line system.

[PRECAUTIONS]

- For medical and other professional *in vitro* diagnostic use only. Do not use 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.
- The used test should be discarded according to local regulations.

[STORAGE AND STABILITY]
 Store as packaged at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch or label of the closed canister. The test must remain in the sealed pouch or closed canister until use. **DO NOT FREEZE.** Do not use beyond the expiration date. NOTE: Once the canister has been opened, the remaining test(s) are stable for 50 days only.

[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 particles should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.

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 before testing.

[MATERIALS]

Materials Provided

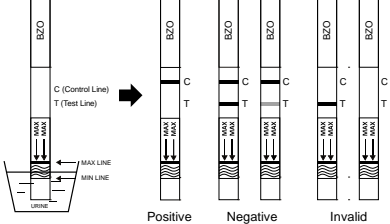
- Package insert

Materials Required But Not Provided

- Specimen collection container
- Timer

[DIRECTIONS FOR USE]
Allow the test, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test dipstick from the sealed pouch and use it within one hour.



- With arrows pointing toward the urine specimen, **immerse the test dipstick vertically in the urine specimen for at least 10-15 seconds.** Do not pass the maximum line (MAX) on the Test Dipstick when immersing the strip. See the illustration below.
- Place the test dipstick on a non-absorbent flat surface, start the timer and wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes

[INTERPRETATION OF RESULTS]
 (Please refer to the illustration above)

NEGATIVE: * **Two lines appear.** One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the Benzodiazepine concentration is below the detectable cut-off level.

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

POSITIVE: **One colored line appears in the control line region (C).** No line appears in the test line region (T). This positive result indicates that the Benzodiazepine concentration exceeds the detectable cut-off level.

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 with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

[QUALITY CONTROL]
 A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

[LIMITATIONS]

- The BZO Rapid Test Dipstick (Urine) 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.^{1,2}
- 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.

[EXPECTED VALUES]
 This negative result indicates that the Benzodiazepines concentration is below the detectable level of 300ng/ml. Positive result means the concentration of Benzodiazepines is above the level of 300ng/ml. The BZO Rapid Test Dipstick has a sensitivity of 300ng/ml

[PERFORMANCE CHARACTERISTICS]

Accuracy
 A side-by-side comparison was conducted using The BZO Rapid Test Dipstick (Urine) and a commercially available BZO rapid test. Testing was performed on 95 clinical specimens previously collected from subjects present for Drug Screen Testing. The following results were tabulated:

Method	Other BZO Rapid Test		Total Results
	Results	Positive	
BZO Rapid Test Dipstick	Positive	43	43
	Negative	0	52
		43	52
Total Results		43	95
% Agreement		>99.9%	>99.9%

A side-by-side comparison was conducted using The BZO Rapid Test Dipstick (Urine) and GC/MS at the cut-off of 300ng/ml. Testing was performed on 250 clinical specimens previously collected from subjects present for Drug Screen Testing. The following results were tabulated:

Method	GC/MS		Total Results
	Results	Positive	
BZO Rapid Test Dipstick	Positive	121	122
	Negative	2	128
		123	250
Total Results		123	250
% Agreement		98.4%	99.2%

Analytical Sensitivity
 A drug-free urine pool was spiked with Oxazepam at the following concentrations: 0ng/ml, 150ng/ml, 225 ng/ml, 300ng/ml, 375ng/ml, 450ng/ml and 900 ng/ml. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Oxazepam Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	27	3
300	Cut-off	30	15	15
375	+25%	30	3	27
450	+50%	30	0	30
900	3X	30	0	30

Analytical Specificity
 The following table lists compounds that are positively detected in urine by the BZO Rapid Test Dipstick (Urine) at 5 minutes.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Alprazolam	100	Flurazepam	200
a-hydroxyalprazolam	1,500	(±) Lorazepam	3,000
Bromazepam	900	RS-Lorazepam glucuronide	200
Chlordiazepoxide	900	Midazolam	6,000
Clobazam	200	Nitrazepam	700
Clonazepam	500	Norchlordiazepoxide	100
Clorazepate dipotassium	500	Nordiazepam	900
Delorazepam	900	Oxazepam	300
Desalkylflurazepam	200	Temazepam	100
Diazepam	300	Triazolam	3,000
Estazolam	6,000		

Precision
 A study was conducted at three hospitals by laypersons using three different lots of product to

demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing, according to GC/MS, no Oxazepam, 25% Oxazepam above and below the cut-off and 50% Oxazepam above and below the 300ng/ml cut-off was provided to each site. The following results were tabulated:

Oxazepam Concentration (ng/mL)	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
150	10	10	0	10	0	10	0
225	10	9	1	9	1	9	1
375	10	1	9	1	9	1	9
450	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity
 Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 150ng/ml and 450ng/ml of Oxazepam. The BZO Test Dipstick (Urine) 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.

Effect of Urinary pH
 The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with Oxazepam to 150ng/ml and 450ng/ml. The spiked, pH-adjusted urine was tested with the BZO Rapid Test Dipstick (Urine) in duplicate. The results demonstrate that varying ranges of pH 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 Oxazepam positive urine. The following compounds show no cross-reactivity when tested with the BZO Rapid Test Dipstick (Urine) at a concentration of 100 µg/ml.

Non Cross-Reacting Compounds		
Acetaminophen	Deoxycorticosterone	MDE
Acetophenetidin	Dextromethorphan	Meperidine
N-Acetylpromacainamide	Diclofenac	Meprbamamate
Acetylsalicylic acid	Diffunisal	Methadone
Aminopyrine	Digoxin	L-Methamphetamine
Amitypyline	Diphenhydramine	Methoxyphenamine
Amobarbital	Doxylamine	(±) - 3,4-Methylenedioxy-amphetamine
Amoxicillin	Ecgonine	(±) - 3,4-Methylenedioxy-methamphetamine
Ampicillin	Ecgonine methylester	Morphine-3-β-D glucuronide
L-Ascorbic acid	(-)y-Ephedrine	D-Pseudoephedrine
D,L-Amphetamine sulfate [R,2S]	(-) Ephedrine	Quinacrine
Apomorphine	(L) - Epinephrine	Morphine Sulfate
Aspartame	Erythromycin	Nalidixic acid
Atropine	β-Estradiol	Naloxone
Benzilic acid	Estrone-3-sulfate	Naltrexone
Benzoic acid	Ethyl-p-aminobenzoate	Naproxen
Benzoylecgonine	Fenoprofen	Niacinamide
Benzphetamine	Furosemide	Nifedipine
Bilirubin	Genistic acid	Norcocaine
(±) - Brompheniramine	Hemoglobin	Norethindrone
Caffeine	Hydralazine	D-Norpropoxyphene
Cannabidiol	Hydrochlorothiazide	Noscapine
Cannabiol	Hydrocodone	D,L-Octopamine
Chlorallyhydrate	Hydrocortisone	Oxalic acid
Chloramphenicol	O-Hydroxyhippuric acid	Oxolinic acid
Chlorothiazide	p-Hydroxyamphetamine	Oxycodone
(±) - Chlorpheniramine	p-Hydroxy-methamphetamine	Oxymetazoline
Chlorpromazine	3-Hydroxytyramine	Papaverine
Chlorquine	Ibuprofen	Penicillin-G
Cholesterol	Imipramine	Pentazocine
Clomipramine	Iproniazid	Perphenazine
Clonidine	(±) - Isoproterenol	Phencyclidine
Cocacethylene	Isoxsuprine	Phenelzine
Cocaine	Ketamine	Phenobarbital
Codine	Ketoprofen	Phentermine
Cortisone	Labetalol	Trans-2-phenylcyclopropylamine hydrochloride
(-) Cotinine	Loperamide	Maprotiline
Creatinine	Meprobamate	Morphine
		β-Phenylethylamine
		Phenylpropanolamine
		Prednisolone
		Prednisone
		Procaine
		Promazine
		Propofol
		D,L-Propanolol
		D-Tetrahydrocannabinol
		Quinidine
		Quinine
		Ranitidine
		Salicylic acid
		Secobarbital
		Serotonin
		Sulfamethazine
		Sulindac
		Tetracycline
		Tetrahydrocortisone, 3-Acetate
		Tetrahydrocortisone 3-(β-D-glucuronide)
		Tetrahydrozoline
		Thiamine
		Thioridazine
		D,L-Tyrosine
		Tolbutamide
		Triamterene
		Trifluoperazine
		Trimethoprim
		Trimipramine
		Tryptamine
		D,L-Tryptophan
		Tyramine
		Uric acid
		Verapamil
		Zemipirac

- [BIBLIOGRAPHY]**
- Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488
 - Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For in vitro diagnostic use only		Use by		Do not reuse
	Store between 2-30°C		Lot Number	REF	Catalog #
	Do not use if package is damaged				

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