

REF 14245-4

4 x 24 mL/9 mL

## BENZODIAZEPINE ENZYME IMMUNOASSAY (BZO)

Wedges each contain usable volumes of 24 mL of R1 reagent and 9 mL of R2 reagent.

### INTENDED USE

The EasyRA Benzodiazepine (BZO) reagent is intended for the qualitative determination of benzodiazepines in human urine at a cutoff value of 200 ng/mL. The assay is designed for prescription use on the EasyRA Clinical Chemistry Analyzer.

**The assay provides a rapid screening procedure for determining the presence of benzodiazepine in urine. The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.**

### SUMMARY AND EXPLANATION OF TEST

Benzodiazepines are a family of sedative-hypnotics (3, 4) that are structurally similar and include widely used drugs such as lorazepam, diazepam, chlordiazepoxide, triazolam, and oxazepam. The different benzodiazepines are absorbed at different rates, and the timing of their psychoactive effects varies with the absorption rate and varied half-lives (5).

Benzodiazepines are prescribed for various conditions including panic disorders (6), insomnia (7), seizures (8), alcohol withdrawal (9), and anxiety (10). However, chronic use of benzodiazepines may lead to tolerance and dependence (11, 12). Benzodiazepines are usually taken orally and are metabolized in the liver via cytochrome p450s (13) or glucuronidation (14). Detection of benzodiazepines or their metabolites in urine can be used as an indication for use of benzodiazepines (15).

### Assay Principle

The EasyRA Benzodiazepine (BZO) reagent is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (16). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, benzodiazepine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody binds to the free drug; the unbound benzodiazepine-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

### REAGENTS PROVIDED

Antibody/Substrate Reagent (R<sub>1</sub>): Contains mouse monoclonal anti-benzodiazepine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R<sub>2</sub>): Contains benzodiazepine-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

### Precautions and Warnings

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).*
- *Do not use the reagents beyond their expiration dates.*

### Instructions for Reagent Handling, Storage and Stability

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date on the label if stored at 2–8°C. Opened reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area.

## SPECIMEN COLLECTION AND HANDLING

Urine samples may be collected in plastic or glass containers. Some plastics may adsorb drugs. Use of plastics such as polyethylene is recommended (17). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2–8°C for up to three days. For longer storage, keep sample frozen at -20°C and then thaw before use. Studies have shown benzodiazepine analytes in urine are stable at -20°C up to six months (18). Samples should be at room temperature (18–25°C) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

## PROCEDURE

### Materials Provided

Medica BZO Reagent Wedge, REF 14245 (Qualitative)

### Additional Materials Required:

Medica EasyCal Benzodiazepine Cutoff Calibrator (Oxazepam, 200 ng/mL), REF 14672

Medica EasyCal Universal Negative Calibrator (Oxazepam, 0 ng/mL), REF 14799

Medica EasyQC Benzodiazepine Negative Control (Oxazepam, 100 ng/mL), REF 14780

Medica EasyQC Benzodiazepine Positive Control (Oxazepam 300 ng/mL), REF 14781

Medica Precision Test Dye Wedge, REF 10764

Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or

Medica Cleaner Wedge – Chemistry, REF 10661

Medica EasyRA Evaporation Caps, REF 10745

### Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Hitachi 717 and on the EasyRA Clinical Chemistry Analyzer.

### Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 200 ng/mL as the cutoff calibrator. Recalibration should be performed after reagent bottle change or if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring of each cutoff level: use the 100 ng/mL and 300 ng/mL controls for the 200 ng/mL cutoff.

## INSTRUCTIONS FOR USE

The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray in the reagent area. Dry the neck of the reagent wedge and check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2. Place Medica EasyRA Evaporation Caps, REF 10745 on both the R1 and R2 openings of the reagent wedge.

**NOTE: Use of the Medica EasyRA Evaporation Cap is required to guarantee on-board calibration stability.**

### Calibration

Medica EasyCal Benzodiazepine Cutoff Calibrator, REF 14672 and Medica EasyCal Universal Negative Calibrator, REF 14799 are required for the calibration of the assay. The calibration interval (30 days maximum) with evaporation caps is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

### Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters or contact Medica Corporation technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

## Results

**Note:** A positive test result does not always mean a person took illegal drugs and a negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

**Qualitative:** The cutoff calibrator, which contains 200 ng/mL of oxazepam, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance ( $\Delta\text{mA}/\text{min}$ ) equal to or greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance ( $\Delta\text{mA}/\text{min}$ ) lower than that obtained with the cutoff calibrator is considered negative.

## Procedural Limitations

1. A positive result from the assay indicates only the presence of benzodiazepines. The test is not intended for quantifying these single analytes in samples.
2. A positive result does not necessarily indicate drug abuse.
3. A negative result does not necessarily mean a person did not take benzodiazepines.
4. Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferences) may influence the urine test result.
5. Positive results should be confirmed by other affirmative, analytical chemical methods (e.g., chromatography), preferably GC/MS or LC/MS.
6. The test is designed for use with human urine only.
7. The test is not for therapeutic drug monitoring.
8. Glucuronide metabolites of oxazepam, lorazepam, and temazepam do not cross-react with the antibodies in this immunoassay. The cross-reactivity of other glucuronide metabolites with this assay is not known.

## Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer and validated on an EasyRA Clinical Chemistry Analyzer.

### Precision:

Qualitative analysis: The five calibrators were evaluated. Typical results ( $\Delta\text{mA}/\text{min}$ ) are as follows:

(mA/min)	Within Run (n=21)			Run-to-Run (n=12)		
	Mean	SD	% CV	Mean	SD	% CV
Negative	361.0	3.1	0.9 %	360.0	2.5	0.7 %
100 ng/mL	417.8	3.5	0.8 %	417.9	2.5	0.6 %
200 ng/mL	455.5	3.5	0.8 %	457.0	1.9	0.5 %
300 ng/mL	483.3	3.5	0.7 %	483.6	2.4	0.6 %
1000 ng/mL	559.7	4.9	0.7 %	561.8	3.3	0.6 %

**Sensitivity:** Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 15 ng/mL.

**Accuracy:** The device as compared to GC/MS or HPLC analysis performed as follows:

200 ng/mL cutoff		GC/MS or HPLC	
		Positive	Negative
EasyRA Benzodiazepine (BZO) reagent	Positive	64	2
	Negative	8	42

The following table summarizes the result for the discordant samples:

<b>Discrepant Specimens – 200 ng/mL</b>	
EasyRA Benzodiazepine (BZO) reagent	Reference Method
-	HPLC 518 ng/mL Lorazepam
-	HPLC 101 ng/mL Demoxepam, 68 ng/mL Oxazepam, 1889 ng/mL Temazepam
-	HPLC 280 ng/mL Lorazepam
-	HPLC 526 ng/mL Lorazepam
-	HPLC 1811 ng/mL Lorazepam
-	GC/MS 286 ng/mL Lorazepam
-	GC/MS 261 ng/mL Alprazolam
-	GC/MS 252 ng/mL Lorazepam
+	HPLC 114 ng/mL Oxazepam
+	GC/MS 185 ng/mL Oxazepam

**Analytical Recovery:** In qualitative analysis, the assay correctly identified spiked samples containing more than 200 ng/mL of oxazepam (n=25, spiked levels or higher than the level 1 control) as positive, and those containing less than 200 ng/mL of oxazepam (n=20, spiked levels less than the level 2 control) as negative.

#### **Specificity<sup>4</sup>**

Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine to various concentrations and evaluated with the assay's calibrated dose response curve.

The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 200 ng/mL benzodiazepine cutoffs or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrators.

#### **Structurally Related Benzodiazepine Compounds:**

Compound	Concentration (ng/mL) at 200 ng/mL
Oxazepam	200
Alprazolam	75
Bromazepam	2100
Chlordiazepoxide	65
Clobazam	750
Clonazepam	65
Diazepam	80
Flunitrazepam	50
Flurazepam	90
Lormetazepam	50
Lorazepam	90
Medazepam	23
Nitrazepam	150
Norfludiazepam	15
Prazepam	75
Temazepam	80
Triazolam	45
Oxazepam-glucuronide	>10,000
Lorazepam-glucuronide	>10,000
Temazepam-glucuronide	>10,000

**Structurally Unrelated Pharmacological Compounds:**

Compound	Concentration (µg/mL) at 200 ng/mL
Acetaminophen	1000
Acetylsalicylic acid	1000
Amitriptyline	180
Amobarbital	1000
Amphetamine	1000
Benzoyllecgonine	1000
Caffeine	1000
Chlorpromazine	200
Cocaine	400
Codeine	1000
Dextromethorphan	1000
Ephedrine	1000
Imipramine	300
Meperidine	600
Methadone	1000
Methamphetamine	1000
Methaqualone	1000
Morphine	1000
Nortriptyline	600
Phemobarbital	1000
Promethazine	1000
Propoxyphene	1000
Secobarbital	1000
Valproic Acid	1000
Lidocaine	1000
Chlorpheniramine	100
Ecgonine	1000
Bupropion	1000
Ranitidine	1000

*It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.*

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## EasyRA Clinical Chemistry Analyzer

<b>Parameters:</b>	<b>Qualitative</b>
Primary Wavelength	340
Secondary Wavelength	N/A
Reaction Type	Qual. Kinetic
Reaction Direction	Increase
Calibration Curve	Increase
Reagent Blank	N/A
Sample Blank	N/A
Reaction Time	2.1 Minutes
On-Board Stability	30 Days
Cal Stability	30 Days

\*with anti-evaporation caps

### Performance Characteristics

The results shown below were obtained with the EasyRA analyzer.

### Inaccuracy/Correlation

Eighty (80) clinical urine specimens were tested qualitatively with the Benzodiazepine Enzyme Immunoassay (EIA) method on the EasyRA. All results were confirmed with an LC/MS method and are summarized in the table below:

<b>EasyRA</b>	<b>(0–200 ng/mL) Negative LC/MS</b>	<b>(&gt;200 ng/mL) Positive LC/MS</b>
Negative (<200 ng/mL)	40	0
Positive (>200 ng/mL)	0	40
<b>% Agreement Negative</b>	100%	
<b>% Agreement Positive</b>	100%	

### Imprecision (CLSI, EP5-A2)

**Qualitative analysis:** Precision of benzodiazepine samples (Negative, 100 ng/mL and Positive, 300 ng/mL) was performed qualitatively (mAbs/min) on one Medica EasyRA Analyzer. Each of the precision samples was analyzed 20 times per run. The mean, standard deviation and the %CV were calculated for within run precision.

### Within-Run Imprecision (EP5-A2)

#### Qualitative Results (Cutoff 200 ng/mL)

Qualitative Results (n=20)

<b>Samples (ng/mL)</b>	<b>Mean (mAbs/Min)</b>	<b>SD (mAbs/Min)</b>	<b>%CV</b>
100	184.0	0.8	0.4%
300	216.6	0.8	0.4%

### % Agreement of Qualitative Precision Results with Target Values

<b>Sample</b>	<b>Acceptance Criteria</b>	<b>Results</b>
100 ng/mL	100%	Negative
300 ng/mL	100%	Positive

**Qualitative analysis:** Two samples of oxazepam were prepared in human urine and analyzed twice a day for 20 days. The samples were tested in qualitative mode and the absorbance change versus time was also measured for each reading. The study followed the protocol defined in EP5-A2. Typical results are as follows:

**Total Imprecision (EP5-A2)**

**Qualitative Results (Cutoff 200 ng/mL)**

Qualitative Results (n=40)

Mean (ng/mL)	SD	%CV
105.7	7.8	7.4%
274.7	9.4	3.4%

**% Agreement of Qualitative Precision Results with Target Values**

Sample	Acceptance Criteria	Results
105.7 ng/mL	100%	Negative
274.7 ng/mL	100%	Positive

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