OraSure Technologies, Inc.

150 Webster Street, Bethlehem, PA 18015-1389
Phone: (610) 882-1820 ■ Fax: (610) 882-1830
www.orasure.com

BENZODIAZEPINES INTERCEPT™ MICRO-PLATE EIA

for use with Intercept™ Drugs of Abuse (DOA) Oral Fluid Specimens

14026 (11/00)

INTENDED USE

The OraSure Technologies, Inc. (OTI) Benzodiazepines Intercept™ MICRO-PLATE EIA is intended for use in the qualitative determination of benzodiazepines in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. **FOR FORENSIC USE ONLY.**

> **The OTI Benzodiazepines Intercept™ MICRO-PLATE EIA provides only a preliminary analytical test result. A more specific alternative chemical method should be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.(1) Clinical consideration and professional judgment should be applied to any drugs of abuse test result, particularly when a preliminary, positive result is observed.**

PRINCIPLE OF THE ASSAY

The OTI Benzodiazepines Intercept™ MICRO-PLATE EIA is a competitive micro-plate immunoassay for the detection of benzodiazepines in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. Specimen or standard is added to an EIA well in combination with an enzyme-labeled hapten derivative. In an EIA well containing an oral fluid specimen positive for benzodiazepines, there is a competition between the drug and the enzyme-labeled hapten to bind the antibody fixed onto the EIA well. EIA wells are then washed, substrate is added, and color is produced. The absorbance measured for each well at 450 nm is inversely proportional to the amount of benzodiazepines present in the specimen or calibrator/control. Because currently there are no SAMHSA assigned cutoffs for benzodiazepines testing using oral fluid, OTI recommends a cutoff of 1.0 ng/mL when testing oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. This cutoff is within the limit of detection by the OTI Benzodiazepines Intercept™ MICRO-PLATE EIA.

PRINCIPLE OF THE INTERCEPT™ DOA ORAL SPECIMEN COLLECTION DEVICE

Saliva is a complex mixture of parotid, submandibular, sublingual and minor salivary gland secretions mixed with mucin, bacteria, leukocytes, sloughed epithelial cells and gingival crevicular fluid. The fact that benzodiazepines are present in oral fluid following human use is well documented.(2,3)

The Intercept™ DOA Oral Specimen Collection Device was developed for the purpose of collecting oral fluid for diagnostic testing. The collection device consists of a treated absorbent cotton fiber pad affixed to a nylon stick (Collection Pad) and a preservative solution in a plastic container (Specimen Vial). The Collection Pad is impregnated with a mixture of common salts and gelatin which creates a hypertonic environment and an increased osmotic pressure wherever it contacts oral mucosal cells. The pad is placed in contact with the gingival mucosa (between the lower gum and cheek) which enhances the flow of mucosal transudate across the mucosal surfaces onto the absorptive cotton fibers of the pad. Following the collection period, the Collection Pad is placed into a vial containing a preservative solution which serves to inhibit the growth of oral micro-organisms recovered on the Collection Pad. The vial is sealed with a plastic cap and transported to a laboratory for processing and testing. Following processing, a fluid containing a mixture of saliva components and the preservative solution is recovered which is

suitable for testing for the presence of benzodiazepines in the OTI Benzodiazepines Intercept™ MICRO-PLATE EIA manufactured by OraSure Technologies, Bethlehem, PA. Refer to the Intercept™ DOA Oral Specimen Collection Device product insert for specific instructions on the proper collection, handling and adequacy of oral fluid samples.

KIT COMPONENTS	Catalog No. 1110IB	Catalog No. 1110IC
	480 Test Kit	9600 Test Kit
	Min. Qty.	Min. Qty.
Micro-Plate - Sheep anti-benzodiazepines polyclonal antibody immobilized on a polystyrene plate.	5	100
Enzyme Conjugate - Horseradish peroxidase labeled with a benzodiazepine hapten and diluted in a protein matrix with stabilizers.	60 mL	1 L
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	60 mL	1 L
Stopping Reagent - Contains 2 N sulfuric acid.	60 mL	1 L
Oral Fluid Negative Calibrator – Oral Fluid Diluent negative for nordiazepam.	2 mL	16 mL
Oral Fluid Negative Control – Oral Fluid Diluent containing 0.5 ng/mL (v/v) of nordiazepam.	2 mL	16 mL
Oral Fluid Cutoff Calibrator – Oral Fluid Diluent containing 1.0 ng/mL (v/v) of nordiazepam.	2 mL	16 mL
Oral Fluid Positive Control – Oral Fluid Diluent containing 2.0 ng/mL (v/v) of nordiazepam.	2 mL	16 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is **NOT** recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do **NOT** mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Do **NOT** add sodium azide to samples as a preservative.
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do **NOT** use reagents past the expiration date.
7. Do **NOT** mix reagents from different kits or manufacturers.
8. Do **NOT** freeze reagents.
9. It is suggested that all OTI reagents be kept out of direct sunlight whenever possible.

STORAGE AND STABILITY OF THE BENZODIAZEPINES INTERCEPT™ MICRO-PLATE EIA

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

INTERCEPT™ DOA SPECIMEN PROCESSING PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Tubes suitable for centrifuging Intercept™ DOA Specimen Vials.
2. Centrifuge capable of 600 - 800 x g.

PROCEDURE (Refer to Intercept™ DOA Collector package insert for more information)

1. Record the specimen identification number from the Intercept™ DOA Specimen Vial.
2. Ensure that the specimen is within acceptable dating for testing, i.e., $\leq$ 21 days from the time of collection.
3. Hold the vial upright with the tip pointed up.
4. Move the pad away from the vial tip by gently tapping the vial.
5. Break the pointed tip of the vial off with your thumb.
6. Place a tube over the vial and invert the tube and vial.

7. Centrifuge at 600 - 800 x g for 15 minutes.
8. Assay or store the resulting eluate according to the procedures described herein.
9. A minimum of 0.7 mL of the eluate sample is required. This can be determined by centrifugation of the samples into graduated containers or by direct pipetting with a calibrated pipet adjusted to the specified volume.
10. If the minimum volume requirement is not met, a new sample should be collected. If this is not possible, a warning should accompany any data generated indicating that an insufficient amount of sample was collected and that this may affect the accuracy of the final result.

ASSAY PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25 and 100 microliters) with tips.
2. Micro-plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.
4. Intercept™ DOA eluate sample(s) – 0.7 mL minimum.

PROCEDURE -- Note: ***Allow all reagents and samples to come to room temperature (15-27°C) before use.***

1. **At the discretion of the operator, all samples, calibrators, and controls may be tested in duplicate. The inclusion of calibrators and controls is recommended in each run.**
2. **Add 25 microliters of sample, calibrator or control to each well. Label wells appropriately.**
3. **Add 100 microliters of Enzyme Conjugate to each test well.**
4. **Incubate for 30 minutes at room temperature (15-27° C) in the dark.**
5. **Using a suitable plate washer, wash each well six (6) times with 300 microliters of distilled water.**
6. **Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (15-27° C) in the dark.**
7. **Add 100 microliters of Stopping Reagent to each well.**
8. **Measure the absorbance at a dual wavelength of 450 and 630 nm within 15 minutes of stopping the reaction.**

INTERPRETATION

Positive Result: Any sample with an absorbance less than or equal to the Oral Fluid Cutoff Calibrator is considered a positive.

Negative Result: Any sample with an absorbance greater than the Oral Fluid Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. Duplicate sample results with a difference of 10% or more should be retested.

QUALITY CONTROL

OTI provides positive and negative controls to monitor the daily performance of the OTI Benzodiazepines Intercept™ MICRO-PLATE EIA. The Oral Fluid Negative Control must have an absorbance greater than the Oral Fluid Cutoff Calibrator, while the Oral Fluid Positive Control must always have an absorbance less than the Oral Fluid Cutoff Calibrator.

LIMITATIONS OF THE PROCEDURE

This assay is designed for use with oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. Other samples may produce variable results, and their use is not recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS OF INTERCEPT™ DOA SPECIMENS

Performance Characteristics have not been determined at this time.

REFERENCES

1. "Urine Testing for Drugs of Abuse," National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Schramm, W., Smith, R.H., and Craig, P.A., "Drugs of Abuse in Saliva: A Review," *Journal of Analytical Toxicology*, 1992; 16:1-9.
3. Cone, E.J., "Saliva Testing for Drugs of Abuse," Presented at the NY Acad. Sciences meeting Oct. 22-25, 1992.
4. Tenovuo, J.O., editor, Human Saliva: Clinical Chemistry and Microbiology, Volume 1 (CRC Press, Boca Raton, FL) 1989.
5. Baselt, R.C. and Cravey, R.H., Disposition of Toxic Drugs and Chemicals in Man (Chemical Tox. Inst., CA), 1995.
6. Intercept™ Oral Specimen Collection Device, Package Insert. Manufactured by OraSure Technologies, Inc., Beaverton, OR 97008.

***Note**: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.*

***