CEDIA® Cannabinoids OFT Assay (EU)

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**
(For Use with Oral-Eze® Oral Fluid Collection System)

REF **10010883 (3 x 18 mL Kit)**
10010888 (65 mL Kit)

Intended Use

The Thermo Scientific CEDIA Cannabinoids OFT Assay is intended for use in the qualitative and semi-quantitative determination of Cannabinoids in human oral fluid at the diluted cutoff concentration of 3.3 ng/mL. The specimen must be collected exclusively with the Oral-Eze® Oral Fluid Collection System. The assay is calibrated against *l*-Δ^9 THC and performed on the Olympus AU680 analyzer. This in vitro diagnostic device is intended for clinical laboratory use only.

The CEDIA® Cannabinoids OFT Assay provides only a preliminary analytical test result. A more specific alternative method must be used to obtain a confirmed analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) are the preferred confirmatory methods.[1-3] Clinical consideration and professional judgment should be applied to any drug of abuse test result particularly when preliminary positive results are used.

Summary and Explanation of the Test

The collection of oral fluid is less invasive and no special facilities are required. Oral fluid contains mostly parent drug and therefore is a better indicator of recent drug use.

Cannabinoids and minor amounts of cannabinoid metabolites could be detected in oral fluid immediately within 30 minutes after administration. Therefore, detection of cannabinoids and their metabolites in oral fluids is a good indicator of recent use.[4, 5] Detection levels and duration of detection of cannabinoids in oral fluid are dependent up on pH and amount of drug consumed.

CEDIA Cannabinoids OFT Assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system.[6] The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously re-associate to form fully active enzyme that, in the assay format, cleave a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment (enzyme donor) of β-galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragment free to form active enzyme. If the analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the re-association of inactive β-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

Reagents

1. **EA Reconstitution Buffer**
   Contains buffer salts, 0.2 mg/L rabbit monoclonal anti-THC antibody, stabilizer and preservative.
1a **EA Reagent**
   Contains 0.171 g/L Enzyme Acceptor (microbial), buffer salts and preservative.
2. **ED Reconstitution Buffer**
   Contains buffer salts, stabilizers, and preservative.
2a **ED Reagent**
   Contains 0.175 nM Enzyme Donor (microbial) conjugated to cannabinoid derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizers, detergent and preservative.

Additional Materials Required (sold separately)

REF	*Kit Description*
10016643	CEDIA THC OFT Negative Calibrator
10016644	CEDIA THC OFT Calibrator 1
10016646	CEDIA THC OFT Calibrator 2
10016647	CEDIA THC OFT Calibrator 3
10016648	CEDIA THC OFT Calibrator 4
10016649	CEDIA THC OFT Control Set
96100-050	Oral-Eze Collection Device (50/Box)
96100-500	Oral-Eze Collection Device (500/Box)
96105-050	Oral-Eze Sample Extractor (50/Box)
96105-500	Oral-Eze Sample Extractor (500/Box)

⚠ Precautions and Warnings

1. This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. Do not use the reagents beyond their expiration dates.

DANGER: Powder reagent contains ≤55% w/w bovine serum albumin (BSA), ≤1% w/w Sodium azide and ≤0.5% w/w Drug-specific antibody (Rabbit). Liquid reagent contains ≤0.5% bovine serum (FBS), ≤0.15% Sodium azide and <0.1% Drug-specific antibody (Rabbit).
H317 – May cause allergic skin reaction.
H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled.
EUH032 – Contact with acids liberates very toxic gases.

Avoid breathing dust/mist/vapors/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/ eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/ attention. If experiencing respiratory symptoms; Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/ container to location in accordance with local/regional/national/international regulations.

Reagent Preparation and Storage

For preparation of the solutions, refer to the section below. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the solutions.

In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, and state regulations.

In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).

Prepare the solutions in the following order to minimize possible contamination.

R2 Enzyme donor solution

Connect Bottle 2a (ED reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at room temperature (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

R1 Enzyme acceptor solution

Connect Bottle 1a (EA reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at room temperature (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.

NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R2 solution (Enzyme Donor) should be yellow-orange in color. A red or red-purple color indicates that the reagent has been contaminated and must be discarded.

NOTE 3: The R1 and R2 solutions must be at the reagent compartment storage temperature of the analyzer before performing the assay. Refer to the analyzer specific application sheet for additional information.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

R1 Solution: 60 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 60 days refrigerated on analyzer or at 2-8°C.

Specimen Collection and Handling

Oral fluid samples are suitable for use in the CEDIA Cannabinoids OFT Assay. Collect oral fluid samples using the Oral-Eze Oral Fluid Collection System. Care should be taken to preserve the chemical integrity of the oral fluid sample from the time it is collected until the time it is assayed by securely capping the samples, storing the samples at 2-8°C or at room temperature (21-25°C), and testing within 21 days after collection.

Handle oral fluid samples as if they were potentially infectious.
Samples within a pH range of 5-9 are suitable for testing with this assay.

Oral-Eze Sample Processing Procedure

1. Label the sample collection vial with proper identification.
2. Check the sample collection date on the vial to ensure that the sample is within 21 days from the date of collection.
3. Open the cap and compress the pad to express the sample.
4. Recap the vial and the sample is ready for testing.
5. Ensure that the oral fluid samples are maintained between 4°C and 37°C during shipping.
6. Samples can be stored at room temperature (21-25°C) for 21 days. They should be stored at 2-8°C.

Assay Procedure

The Oral-Eze Oral Fluid Collection Device contains a preservative buffer that dilutes the neat oral fluid sample. The calibrator and control levels are set at diluted levels.

NOTE: To correlate the Oral-Eze result from the assay or the associated LC-MS/MS confirmation result to a neat oral fluid value, the result from the Oral-Eze sample should be multiplied by a factor of 3.

1. Pipet the processed oral fluid samples and controls into labeled sample cups and place the cups into the sample ring of the Olympus AU680 analyzer.
2. Load reagents (reagent 1 and reagent 2) into the reagent compartment of the analyzer.
3. Pipet calibrators into labeled cups and load the cups into the sample rack of the analyzer.
4. Program the run setup using 540 nm as primary wavelength and 660 nm as the secondary wavelength. Refer to the parameter sheet for detailed instructions on how to program the analyzer.

Quality Control and Calibration

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. Recalibrate the test if reagents are changed or if control results are outside of established limits. Ensure that control results are within the established range, as determined by laboratory procedures and guidelines. If results fall outside of the established ranges, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements. Each laboratory should establish its own calibration and control frequency.

Results and Expected Values

Qualitative Results

The cutoff calibrator (calibrator 2) is used as reference in distinguishing "positive" from "negative" samples. Samples producing a response value equal to or greater than the response value of the cutoff calibrator are considered positive. Samples producing a response value less than the response value of the cutoff calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

Semi-quantitative Results

A standard curve is run using all the calibrators to estimate relative concentrations of the drug in the samples. Refer to the analyzer specific application sheet for detailed information.

Limitations

A positive result from this assay indicates only the presence of cannabinoids and does not necessarily correlate with the extent of physiological and psychological effects.

It is possible that other substances and/or factors (e.g.: technical or procedural), other than those investigated in the specificity study may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance results obtained on the Olympus AU680 analyzer are shown below. The results obtained in your laboratory may differ from these data.

Precision

The samples containing various amounts of *l*-isomer Δ^9 THC were tested in qualitative and semi-quantitative modes using CLSI (EP05-A2) precision protocol. The samples were randomized and tested in replicates of six, twice a day for four days, total N=48. The results are summarized in the table below.

Qualitative Analysis:

Samples Conc	Result Mean (mA/min)	Within-Run		Total Run	
		SD	%CV	SD	%CV
Low Control	359	4.0	1.1	6.5	1.8
Cutoff Calibrator	414	4.7	1.1	7.8	1.8
High Control	484	6.2	1.3	9.2	1.9

Semi-quantitative Analysis:

Samples	Result Mean (ng/mL)	Within-Run		Total Run	
		SD	CV%	SD	CV%
Low Control	2.0	0.1	6.7	0.3	14.7
Cutoff Calibrator	3.5	0.1	3.2	0.2	6.8
High Control	5.6	0.1	2.6	0.3	5.0

Specificity and Cross-Reactivity

Cannabinoids compounds and metabolites were tested for cross-reactivity in the assay. The potential cross-reactant compound was spiked into a negative oral fluid at the listed concentrations and tested in qualitative and semi-quantitative mode. Then concentrations listed below produced a result approximately equal to the cutoff calibrator.

Compounds	Tested Concentration (ng/mL)	Cannabinoids OFT Assay Negative / Positive
l-11-nor- Δ^9 THC-COOH	3.5	Positive
11-OH- Δ^9 THC	4.0	Positive
Δ^8 THC	6.0	Positive
Cannabinol	6.25	Positive
Cannabidiol	11000	Positive

Various common over-the-counter medications and structurally unrelated compounds were tested for cross-reactivity in the assay. The cross-reactant solutions were prepared by adding the compound to negative oral fluid at the concentrations listed in the table below. All the compounds tested negative and did not show any cross-reactivity.

Compounds	Tested Concentration (ng/mL)	Cannabinoids OFT Assay Negative/Positive
Acetaminophen	80,000	Negative
Acetylsalicylic Acid	80,000	Negative
Alprazolam	10,000	Negative
Amobarbital	10,000	Negative
Amoxicillin	80,000	Negative
Amphetamine	80,000	Negative
Ampicillin	10,000	Negative
Atropine	10,000	Negative
Benzoylecgonine	40,000	Negative
Butabarbital	10,000	Negative
Butalbital	10,000	Negative
Caffeine	8,000	Negative
Captopril	40,000	Negative
Chlorpromazine	10,000	Negative
Chorazepate	10,000	Negative
Chordiazepoxide	8,000	Negative
Cimetidine	40,000	Negative
Clonazepam	10,000	Negative
Cocaethylene	10,000	Negative
Cocaine	500	Negative
Codeine	80,000	Negative
Cyclizine	10,000	Negative
Dextromethorphan	80,000	Negative
Diazepam	40,000	Negative
Digoxin	8,000	Negative
Diphenhydramine	10,000	Negative
Enalapril	40,000	Negative
Fluoxetine	40,000	Negative
Gentisic Acid	10,000	Negative
Hydrocodone	10,000	Negative
Hydromorphone	10,000	Negative
Ibuprofen	40,000	Negative
Imipramine	10,000	Negative
l-Ephedrine	10,000	Negative
Levothyroxine	4,000	Negative
Lidocaine	10,000	Negative
Loperamide	10,000	Negative
Medazepam	10,000	Negative
Meperidine	80,000	Negative
Methadone	80,000	Negative
Methamphetamine	80,000	Negative
Metoprolol	10,000	Negative
Morphine	16,000	Negative
Naproxen	80,000	Negative
Niacinamide	10,000	Negative
Nifedipine	40,000	Negative
Norchlordiazepoxide	10,000	Negative
Oxazepam	40,000	Negative
Penicillin	10,000	Negative

(Con't)

Compounds	Tested Concentration (ng/mL)	Cannabinoids OFT Assay Negative/Positive
Phencyclidine	80,000	Negative
Phenethylamine	10,000	Negative
Phenobarbital	80,000	Negative
Phenylepherine	10,000	Negative
Phenylpropanolamine	10,000	Negative
Procainamide	10,000	Negative
Procaine	10,000	Negative
Propoxyphene	80,000	Negative
Pseudoephedrine	10,000	Negative
Quinidine	10,000	Negative
Ranitidine	40,000	Negative
Salbutamol	10,000	Negative
Salicyluric Acid	40,000	Negative
Secobarbital	80,000	Negative
Temazepam	10,000	Negative
Theophylline	10,000	Negative
Tolmetin	40,000	Negative
Verapamil	40,000	Negative
Zomepirac	10,000	Negative

Endogenous, Exogenous Substances and pH Interference

The potential interference from several endogenous and exogenous substances, and pH on the detection accuracy of the samples containing *l*-isomer Δ^9 THC at the low and high control concentrations were tested in the assay. The interfering substances were added to the negative oral fluid at the concentrations listed in the table below. The samples were tested in both qualitative and semi-quantitative modes. No interference was observed with the interfering substances and pH samples at the low and high controls concentrations.

Substances	Tested Concentration (ng/mL)	Cannabinoids OFT Assay	
		Low Control	High Control
Low Control	1.7	Negative	N/A
High Control	5.0	N/A	Positive
Cotinine	0.01 mg/mL	Negative	Positive
Nicotine	0.01 mg/mL	Negative	Positive
Hemoglobin	0.10 mg/mL	Negative	Positive
Human Serum Albumin	2.5 mg/mL	Negative	Positive
Sodium Chloride	6.0 mg/mL	Negative	Positive
Cholesterol	0.15 mg/dL	Negative	Positive
Acetaminophen	0.1 mg/mL	Negative	Positive
Acetylsalicylic Acid	0.1 mg/mL	Negative	Positive
Caffeine	0.1 mg/mL	Negative	Positive
Ibuprofen	0.1 mg/mL	Negative	Positive
Coffee	2% v/v	Negative	Positive
Milk	0.5 % v/v	Negative	Positive
Orange Juice	2% v/v	Negative	Positive
Cranberry Juice	2% v/v	Negative	Positive
Soft drink (Coke)	2% v/v	Negative	Positive
Toothpaste	2% v/v	Negative	Positive
Mouthwash	2% v/v	Negative	Positive
Tea	2% v/v	Negative	Positive
Denture Adhesive	2% v/v	Negative	Positive
pH	5-9	Negative	Positive

Method Comparison

Forty-one oral fluid samples from rehabilitation clinic were collected using the Oral-Eze Oral Fluid Collection Device. The oral fluid samples were tested in the CEDIA Cannabinoids OFT Assay and by LC-MS/MS method.

Qualitative Analysis: The overall concordance between CEDIA Cannabinoids OFT Assay and LC-MS/MS using a cutoff of 3.3 ng/mL is 98.0%. The comparision of sample results by the CEDIA Cannabinoids OFT Assay to LC-MS/MS showed 100.0% sensitivity and 95.0% specificity.

Qualitative

		LC-MS/MS (ng/mL)	
		+	-
CEDIA	+	20	1*
	-	0	20

*Discordant sample was borderline Negative by LC-MS/MS.

Semi-quantitative Analysis: The overall concordance between CEDIA Cannabinoids OFT Assay and LC-MS/MS using a cutoff of 3.3 ng/mL is 100.0%. Then comparision of sample results by the CEDIA Cannabinoids OFT Assay to LC-MS/MS showed 100.0% sensitivity and 100.0% specificity.

Semi-quantitative

		LC-MS/MS (ng/mL)	
		+	-
CEDIA	+	20	0
	-	0	21

References

1. Cone, E. J., et al. (2002). Oral Fluid Testing for Drugs of Abuse: Positive Prevalence Rates by Intercept (TM) Immunoassay Screening and GC-MS-MS Confirmation and Suggested Cutoff Concentrations. *Journal of Analytical Toxicology*, 26, 541-547.
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