Cannabinoids ELISA Kit

Catalog Number KA0928
96 assays
Version: 06

Intended for research use only
# Table of Contents

**Introduction** ..................................................................................................................... 3  
- Intended Use ......................................................................................................................... 3  
- Background ........................................................................................................................... 3  
- Principle of the Assay ........................................................................................................... 3  

**General Information** ............................................................................................................ 4  
- Materials Supplied ............................................................................................................... 4  
- Storage Instruction ............................................................................................................... 4  
- Materials Required but Not Supplied ................................................................................... 4  
- Precautions for Use ............................................................................................................. 5  

**Assay Protocol** ..................................................................................................................... 6  
- Sample Preparation .............................................................................................................. 6  
- Assay Procedure .................................................................................................................. 6  

**Data Analysis** ...................................................................................................................... 7  
- Calculation of Results ......................................................................................................... 7  
- Performance Characteristics ................................................................................................. 7  

**Resources** ............................................................................................................................ 9  
- Reference ............................................................................................................................. 9  
- Plate Layout .......................................................................................................................... 10
Introduction

Intended Use

The Cannabinoids ELISA Kit provides only a 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 (GS-MS) is the preferred confirmatory method (1). Professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

The Cannabinoids ELISA Kit is a specific and sensitive in-vitro test to detect the presence of cannabinoids in samples such as whole blood, serum, plasma and urine.

Background

THC (a member of the cannabinoid family) is the primary psychoactive ingredient of marijuana (1). Cannabinoid metabolites appear in urine two to four hours after a marijuana smoke and may persist for days (up to thirty) (1-3). Thus a urine assay reasonably serves to detect cannabis use even through a considerable period may have elapsed since smoking or ingestion of marijuana.

Principle of the Assay

The Cannabinoids ELISA Kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. A 10 μL aliquot of a diluted unknown specimen is incubated with a 100 μL dilution of enzyme (Horseradish peroxidase) labeled carboxy THC (THCA) derivative in micro-plate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 1 ng/mL. The Cannabinoids ELISA Kit avoids extraction of urine or blood sample for measurement. It employs a polyclonal high affinity, purified carboxy THC antibody. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding protein(s) or other macromolecules.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with polyclonal anti-carboxy THC</td>
<td>96 (8x12) wells</td>
</tr>
<tr>
<td>THC conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Immunalysis Positive Ref. Std</td>
<td>2 mL</td>
</tr>
<tr>
<td>Neg Std.</td>
<td>1 mL</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>15 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>15 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

✓ Store the kit at 2-4°C.
✓ Keep microwells sealed in a dry bag with desiccants.
✓ The reagents are stable until expiration of the kit.
✓ Do not expose reagents to heat, sun, or strong light.

Materials Required but Not Supplied

✓ Distilled or deionized water
✓ Precision pipettes
✓ Disposable pipette tips
✓ ELISA reader capable of reading absorbance at 450 nm
✓ Absorbance paper or paper towel
✓ Graph paper
Precautions for Use

This test kit is designed for Research Use Only.

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984

✓ Do not add sodium azide to samples as preservative. Do not use external controls containing sodium azide.

✓ Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

✓ The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

✓ It is recommended that serum samples be run in duplicate.

✓ Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
**Assay Protocol**

**Sample Preparation**

1. **Precautions:** The Cannabinoids ELISA Kit is to be used with human samples such as whole blood, serum, urine and plasma. Abnova has not tested all possible applications of this assay. The Cutoff criteria are important in deciding the sample dilution. It is recommended to dilute most blood samples either 1:5 or 1:10 depending on the cutoff used by the laboratory.

2. **Additives:** Specimens to which sodium azide has been added affect the assay.

3. **Storage and Handling Instructions:** Urine samples should be stored at 2-4°C until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

**Assay Procedure**

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (Urine samples are normally diluted 1:10 for a THCA cutoff of 50 ng/mL.) The dilution factor and volume added can be adjusted based on the laboratory's cutoff.

2. Add 10 μL of appropriately diluted standards to each well in duplicate.

3. Add 10 μL of the diluted specimens in duplicate (recommended) to each well.

4. Add 100 μL of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.

5. Incubate for 60 minutes at room temperature (20-25°C) preferably in the dark, after addition of enzyme conjugate to the last well.

6. Wash the wells 6 times with 350 μL distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples, containing abnormally high amounts of hemoglobin (some Postmortem samples), use 10 mM Phosphate buffered saline pH 7.0-7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.

7. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate, does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.

8. Add 100 μL of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.

9. Incubate for 30 minutes at room temperature, preferably in the dark.

10. Add 100 μL of Stop Solution to each well, to change the blue color to yellow.

11. Measure the absorbance at a dual wavelength of 450 nm and 650 nm.

12. Wells should be read within 1 hour of yellow color development.
Data Analysis

Calculation of Results

If the average sample absorbance is equal to or less than the average absorbance of the laboratory THCA/CTHC positive reference standard the sample is POSITIVE for cannabinoids. If the average sample absorbance is greater than the average absorbance of the laboratory THCA/CTHC positive reference standard the sample is called NEGATIVE for cannabinoids. Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve.

✓ The following data represent a typical dose/response curve.

<table>
<thead>
<tr>
<th>CTHC ng/mL</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.985</td>
</tr>
<tr>
<td>2</td>
<td>1.413</td>
</tr>
<tr>
<td>5</td>
<td>0.955</td>
</tr>
<tr>
<td>10</td>
<td>0.751</td>
</tr>
</tbody>
</table>

The dose/response curve shown above should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or a cutoff calibrator should be run with every plate.

Performance Characteristics

✓ Accuracy

35 whole blood samples and 60 urine samples collected from presumed non-users were tested in the Cannabinoids ELISA Kit. One hundred percent of these normal samples measured negative at 20 ng/mL of THCA equivalents for whole blood and 50 ng/mL of THCA equivalents for urine. Forty whole blood samples which were previously confirmed positive for cannabinoids by GC-MS employing a cut-off of 10 ng/mL THCA were tested in the Cannabinoids ELISA Kit. All the samples were found to be positive i.e. above the cut-off of 20 ng/mL.

✓ Precision

The precision of the Cannabinoids ELISA Kit has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.
**Intra-assay Precision**

Intra-assay precision was determined with reference controls. A 0, 2, 5 and 10 ng/mL standard was assayed eight times in the same assay.

<table>
<thead>
<tr>
<th>carboxy THC (ng/mL)</th>
<th>Mean Abs.</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.905</td>
<td>0.139</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>1.114</td>
<td>0.103</td>
<td>9.4</td>
</tr>
<tr>
<td>5</td>
<td>0.752</td>
<td>0.066</td>
<td>8.8</td>
</tr>
<tr>
<td>10</td>
<td>0.549</td>
<td>0.042</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Sensitivity**

Assay sensitivity based on the minimum THCA concentration required to produce a four standard deviation from assay zero dose response (Ao) is 1 ng/mL.

**Specificity**

The specificity of Cannabinoids ELISA Kit for was determined by generating inhibition curves for each of the compounds listed below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>approx. ng/mL equivalent to 10 ng THCA/mL</th>
<th>Cross-reactivities</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-nor-9 carboxy THC</td>
<td>11</td>
<td>110</td>
</tr>
<tr>
<td>9^-THC</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>THC</td>
<td>22</td>
<td>45</td>
</tr>
<tr>
<td>11-hydroxy^-THC</td>
<td>&gt;1000</td>
<td>&lt;5</td>
</tr>
<tr>
<td>8-11-Dihydroxy^-THC</td>
<td>&gt;1000</td>
<td>&lt;5</td>
</tr>
<tr>
<td>cannabidiol</td>
<td>&gt;1000</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

**Cross-Reactivity with Unrelated Drugs**

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 2,000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level. Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbital, Butabarbital, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chloropromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estradiol, Estrone, Estradiol, Ethothoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytoin, Methyl--propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Noradrenaline, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenthiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline.
Resources

Reference

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>