Product Information

Cannabinoids (THCA/CTHC) ELISA Kit

Catalog Number SE120020
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The Cannabinoids Direct 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. Δ⁹-THC (a member of the cannabinoid family) is the primary psychoactive ingredient of marijuana. Cannabinoid metabolites appear in urine two to four hours after exposure to marijuana smoke and may persist for days (up to thirty). Thus a urine assay reasonably serves to detect cannabis use even though a considerable period may have elapsed since smoking or ingestion of marijuana.

The Cannabinoids Direct ELISA Kit is based upon the competitive binding to an 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 microplate 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 THC Direct 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 microplate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

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. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
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<tbody>
<tr>
<td>Microwell coated with polyclonal anti-carboxy THC</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>THC-Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Immunalysis Positive Reference Standard</td>
<td>2 mL</td>
</tr>
<tr>
<td>Negative Standard</td>
<td>1 mL</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Reagent</td>
<td>11 mL</td>
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</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
**Preparation Instructions**

**Sample Preparation**

1. **Precautions** – The Cannabinoids ELISA Kit is to be used with human samples such as whole blood, serum, urine, and plasma. All possible applications of this assay have not been tested. 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 ice pack or equivalent.

**Storage/Stability**

Store the kit at 2–8°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.

**Procedure**

**Notes:** 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 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.

Bring all specimens and kit reagents to room temperature (18–26°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 cutoff used in the laboratory.
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 (18–26°C) preferably in the dark, after addition of Enzyme Conjugate to the last well.
6. Wash the wells 6 times with 350 µL of 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 TMB 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.
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>
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<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>
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</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.

References