REFERENCES

Cannabinoids (THCA/CTHC) ELISA Kit
Catalog No. GWB-BQK10C (96 Tests)

INTENDED USE
For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED
1. Microwell coated with polyclonal anti-carboxy THC 12x8x1
2. THC-Conjugate 12 ml
3. Immunalysis Positive Reference Standard 2 ml
4. Negative Standard 1 ml
5. TMB Substrate 12 ml
6. Stop Reagent 11 ml

MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

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

WARNINGS AND PRECAUTIONS
1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

**SPECIMEN COLLECTION**

1. **Precautions**
   The Cannabinoids Direct ELISA Kit is to be used with human samples such as whole blood, serum, urine and plasma. 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.

**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.