D9-Tetrahydrocannabinol

ELISA Quantitative

Catalog # 19D9THC01-96

www.hygiena.com

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Quantitative ELISA

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For the quantitative detection of D9-Tetrahydrocannabinol in cannabis flowers, concentrates, and edibles and hemp products.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

Contents

Introduction

The rapidly growing cannabis industry has resulted in the proliferation of state regulations to ensure the quality and safety of cannabis products. For example, suppliers must provide potency information regarding the level of total tetrahydrocannabinol (THC) in their products. THC levels can vary tremendously depending on the strain, cultivation technique, and the part of the plant that is utilized. Thus, it's critical to supply accurate THC information to end users for the purpose of dosing correctly.

Several methods for potency measurement have been developed, including gas chromatography, and high pressure liquid chromatography (HPLC), which are reliable and accurate for quantification of specific compounds. However, there are drawbacks. For example, HPLC is time intensive, requires sample clean-up, advanced training, and can be costly.

The Hygiena D9-THC ELISA was developed to measure total THC in a variety of cannabis products in a high-throughput manner. The standard curve is very sensitive with a range of 500-8000 ng/mL. The D9-THC ELISA is capable of measuring %THC in a variety of cannabis products by altering the dilution to meet different %THC ranges, thus offering flexibility to meet variance among cannabis products. The Hygiena D9-THC ELISA may be useful to the cannabis value chain from growers to manufacturers of edibles who need rapid, cost-effective screening methods for measuring total THC.

Assay Principle

The Hygiena D9-THC Assay is a solid phase competitive enzyme immunoassay. An antibody with a high affinity for D9-THC is coated onto polystyrene microwells. Standard or sample is mixed with D9-THC-HRP conjugate and added to the appropriate well. If D9-THC is present in the standard or sample, it will compete with the D9-THC-HRP for binding to the coated antibody. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate is added which develops a blue color in the presence of an enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of D9-THC in the standard or sample. Therefore, as the concentration of D9-THC in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm (OD450). The optical densities of the samples are compared to the ODs of the kit standards and an interpolated result is determined.

Limitations Of The Procedure

- For research use only. Not for use in diagnostic procedures.
- Bring all reagents to room temperature (19° - 25°C) before use.
- Store reagents at 2°C to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
- Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.

Precautions For Users

- Never pipette reagents or samples by mouth.
- The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
- Standards are prepared in methanol, which is flammable. Keep reagents away from heat sources. Wear protective gloves when using this kit.
- Dispose of all materials, containers and devices in an appropriate receptacle after use.
- HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

Reagents Provided

1X Pouch	**Antibody coated microwell plate**		96 wells (12 eight well holder) coated with a mouse anti-D9-THC monoclonal antibody, *Ready-To-Use*.
1X Plate	**Mixing wells**	Red	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-To-Use*.
6X Vials	**D9-THC standards**	Black cap	1.0 mL/vial of D9-THC at the following concentrations: 0, 500, 1000, 2000, 4000, 8000 ng/mL (ppb), *Ready-To-Use*.
1X Vial	**100X D9-THC HRP- conjugate**	Amber cap	0.25 mL of D9-THC conjugated to horseradish peroxidase in buffer with preservative. Dilute conjugate into conjugate buffer to 1X prior to use.
2X Bottle	**Conjugate diluent**	Green cap	2 x 12 mL of conjugate diluent buffer for dilution of 100X conjugate, *Ready-To-Use*.
1X Bottle	**Substrate reagent**	Blue cap	12 mL stabilized tetramethylbenzidine(TMB), *Ready-To-Use*.
1X Bottle	**Stop solution**	Red cap	12 mL Acidic Solution, *Ready-To-Use*.
1X Pouch	**Washing buffer**		PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.

Materials Required But Not Provided

- Single or multi-channel pipettor with 100, 200, and 1000 µL tips
- Microtubes
- Timer
- Wash bottle
- Absorbent paper towels
- Centrifuge
- Vortex mixer
- Blender
- Methanol (MeOH) and/or acetonitrile (Acn)
- Distilled water
- Dry ice
- Water bath that reaches 50°C
- -80°C freezer
- Microplate reader equipped with a 450 nm filter

Preparation of Cannabis Samples

Note: The sample must be collected according to the appropriate established sampling techniques.

Flower

1. Place a bottle of MeOH into the -80°C to chill the solvent overnight.
2. Weigh 0.5-1 g of whole flower. Perform the decarboxylation procedure to convert acid tetrahydrocannabinol to the psychoactive form, D9-Tetrahydrocannabinol. The procedure selected by the user should be validated to ensure full decarboxylation.
3. Add chilled MeOH at a volume that is 40-fold greater than the weight of the sample (i.e. for 1 g of sample, add 40 mL of methanol).
4. Blend the mixture for a total of 2min in an explosion proof blender.
5. Centrifuge the sample at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
6. Dilute the supernatant at 1:10 in 70% MeOH. For example, add 100µL of supernatant to 900 µL of 70% MeOH. Vortex briefly.
7. Dilute this mixture by an additional 1:100 by combining 10 µL of supernatant to 990 µL of 70% MeOH. Vortex briefly.
8. The sample is now ready for testing. The total dilution factor is 1:40,000.

Concentrate

1. Place a bottle of MeOH into the -80°C to chill the solvent overnight.
2. If necessary, perform the decarboxylation procedure on the sample to convert acid tetrahydrocannabinol to the psychoactive form, D9-Tetrahydrocannabinol. The procedure used should be validated by the user to ensure full decarboxylation.
3. Transfer 0.1-0.25 g of sample to a clean vessel. Add chilled MeOH at a volume that is 50-fold greater than the weight of the sample (i.e. for 0.1 g of sample, add 5 mL of MeOH).
4. Vortex the sample for 2min or until the concentrate appears to be completely dissolved in the solvent.
5. Centrifuge the sample at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
6. Dilute the supernatant to solvent at 1:2500 in 70% MeOH with a step wise dilution. For example, first perform a 1:25 dilution by adding 40 µL of sample to 960 µL of 70% MeOH. Do a second dilution at 1:100 by adding 10 µL of the 1:25 dilution into 990 µL of 70% MeOH. Vortex briefly.
7. The sample is now ready for testing. The total dilution factor is 1:125,000.

Preparation of Cannabis Samples Continued

Cookie

1. Place a bottle of MeOH into the -80°C to chill the solvent overnight.
2. Freeze the entire cookie sample on dry ice and grind in a blender.
3. Weigh 5-10 g of cookie sample into a clean blender vessel.
4. Add chilled MeOH at a volume that is 5-fold greater than the weight of the sample (i.e. for 10 g of sample, add 50 mL of MeOH)
5. Blend the mixture for a total of 2min in an explosion proof blender.
6. Centrifuge a portion of the sample at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
7. Dilute the supernatant at 1:40 in 70% MeOH. For example, add 20 µL of supernatant to 780 µL of 70% MeOH. Vortex briefly.
8. The sample is now ready for testing. The total dilution factor is 1:200.

Gummy

1. Prepare a waterbath at 50°C.
2. Weigh 5-10 g of gummy sample into a clean heat proof vessel.
3. Add MeOH at a volume that is 5-fold greater than the weight of the sample (i.e. for 10 g of sample, add 50 mL of MeOH) and place in the water bath for 20min. Occasionally vortex the sample during incubation to ensure mixing. Note: The entire sample may not completely dissolve.
4. Vortex to mix and transfer a portion to a small tube and centrifuge at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
5. Dilute the supernatant 1:50 in 70% MeOH. For example, add 20 µL of supernatant to 980 µL of 70% MeOH. Vortex briefly.
6. The sample is now ready for testing. The total dilution factor is 1:250.

Beverage

1. Invert the beverage container several times to ensure the sample is even.
2. Transfer 100 µL of sample to a clean container.
3. Add 9.9 mL of 70% MeOH and vortex for 2min.
4. The sample is now ready for testing. The total dilution factor is 1:100.

Preparation of Cannabis Samples Continued

Chocolate

1. Prepare a waterbath at 50°C.
2. Weigh 5-10 g of chocolate sample into a clean heat proof vessel.
3. Add Acn at a volume that is 5-fold greater than the weight of the sample (i.e. for 10 g of sample, add 50 mL of Acn) and place in the water bath for 20min. Occasionally vortex the sample during incubation to ensure mixing. Note: The sample may appear clumpy, which is fine.
4. Vortex to mix and transfer a portion to a small tube and centrifuge at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
5. Dilute the supernatant at 1:60 in 70% MeOH. For example, add 15 µL of supernatant to 885 µL of 70% MeOH. Vortex briefly.
6. The sample is now ready for testing. The total dilution factor is 1:300.

Preparation of Hemp Samples

Hemp Seed

1. Weigh 5-10 g of hemp seed into a clean vessel. Perform the decarboxylation procedure to convert acid tetrahydrocannabinol to the psychoactive form, D9-Tetrahydrocannabinol.
2. Add MeOH at a volume that is 15-fold greater than the weight of the sample (i.e. for 10 g of sample, add 150 mL of MeOH).
3. Blend the mixture for a total of 2min in an explosion proof blender.
4. Centrifuge the sample at 3,500rpm for 5min. Carefully transfer the supernatant into a clean tube.
5. Dilute the supernatant at 1:100 in 70% MeOH. For example, add 10µL of supernatant to 990 µL of 70% MeOH. Vortex briefly.
6. The sample is now ready for testing. The total dilution factor is 1:1500.

Preparation of Hemp Samples Continued

Hemp Oil

1. Pipet 1 mL of hemp oil into a clean vessel. Perform the decarboxylation procedure to convert acid tetrahydrocannabinol to the psychoactive form, D9-Tetrahydrocannabinol.
2. Add MeOH at a volume that is 15-fold greater than the volume of the sample (i.e. for 1 mL of sample, add 15 mL of MeOH).
3. Vortex or mix the sample for 15min.
4. Centrifuge the sample at 3,500rpm for 5min. The oil layer should separate out to the bottom. Carefully transfer the supernatant into a clean tube.
5. Dilute the supernatant at 1:100 in 70% MeOH. For example, add 10µL of supernatant to 990 µL of 70% MeOH. Vortex briefly.
6. The sample is now ready for testing. The total dilution factor is 1:1500.

Hemp Oil Capsule and Gummy

1. Transfer 5-10g of sample to a vessel and freeze on dry ice.
2. Blend the sample with a grinder.
3. Add MeOH at a volume that is 15-fold greater than the weight of the sample (i.e. for 1 g of sample, add 15 mL of MeOH). Blend the mixture for a total of 2min in an explosion proof blender.
4. Centrifuge the sample at 3,500rpm for 5min. The oil layer or precipitate should separate out to the bottom. Carefully transfer the supernatant into a clean tube.
5. Dilute the supernatant at 1:100 in 70% MeOH. For example, add 10µL of supernatant to 990 µL of 70% MeOH. Vortex briefly.
6. The sample is now ready for testing. The total dilution factor is 1:1500.

Assay Procedure

1. Bring the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container.
2. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place twice the number of Antibody Coated Microtiter Wells in another microwell holder to run duplicates.
3. Place a clean microtube into a rack for each standard and sample to be tested. Aliquot 400 µL of PBS-Tween wash buffer into microtubes. Using a fresh pipet tip, transfer 100 µL of standard or sample to each microtube. Mix by vortexing.
4. Determine the required volume of conjugate to prepare. For each standard and sample to be run in duplicate, 220 µL of conjugate is needed. Prepare conjugate at 1X working concentration by dilution into conjugate dilution buffer. For example, for 6 standards and 2 samples to run in duplicate, prepare 220 µL x 8 = 1760 µL of conjugate. For this, combine 17.6 µL of 100X conjugate with 1742.4 µL of conjugate buffer. Mix by vortexing.
5. Transfer 200 µL of conjugate into each mixing well. Transfer 50 µL of standard and sample from step#3 into each mixing well. Use a multichannel pipettor to mix by pipetting up and down, then transfer 100 µL of standards and samples from the mixing well into the appropriate Antibody Coated Wells in duplicate. If running singlets, scale the volume down accordingly.
6. Incubate at ambient temperature (19º - 25ºC) for 15 minutes.
7. Decant the contents from the microwells into a discard basin. Wash the wells by filling with the reconstituted PBS-Tween wash buffer, then decanting the buffer into the discard basin. Repeat for a total of five washes. Tap the wells (face down) on a layer of absorbent paper to remove residual wash buffer.
8. Add 100 µL of enzyme substrate (TMB) to each well and incubate for 10 minutes. Cover to avoid direct light (TMB substrate is light sensitive).
9. Stop the reaction by adding 100 µL stop solution. The blue color will change to yellow.
10. Read the optical density (OD) of each microwell with a microplate reader at 450 nm using an air blank or a differential filter of 630 nm.

Interpretation of Results

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/Bo) of the OD of the zero (0.0) standard. Unknowns are measured by interpolation from the standard curve. We recommend using 4PL analysis.

The information contained on the label of each standard vial refers to the contents of that vial. However, the sample has been diluted by extraction solvent as instructed in the EXTRACTION PROCEDURE and so the level of THC shown by the standard must be multiplied by 100, 200, 250, 300, 40,000, or 125,000 in order to indicate the ng per gram (ppb) of the commodity. To convert this value, divide the ng/mL quantity to obtain µg/mL. Convert this further by dividing the µg/mL quantity by 1000 to obtain mg/mL. Covert this further by dividing the mg/mL quantity by 1000 to obtain g/mL. This is equivalent to g/g. To obtain %THC, multiply the g/g quantity by 100%.

For example, a cookie was extracted and tested, which gave an interpolated value of 5,000,000 ng/mL.

$$\frac{5{,}000{,}000\ \text{ng THC}}{\text{mL}} \times \frac{1\ \text{ug}}{1{,}000\ \text{ng}} \times \frac{1\ \text{mg}}{1{,}000\ \text{ug}} \times \frac{1\ \text{g}}{1{,}000\ \text{mg}} = \frac{0.005\ \text{g THC}}{\text{mL}} = \frac{0.005\ \text{g THC}}{\text{g}}$$

$$\frac{0.005\ \text{g THC}}{\text{g}} \times 100\ \% = 0.5\ \%\,\text{THC}$$

The Hygiena D9-THC ELISA is highly sensitive and can detect the varying ranges of THC in a variety of strains and cannabis products. The %THC detectable for each commodity based on the final dilutions are:

Standard (ng/mL)	Flower (1:40k) %THC	Concentrate (1:125k) %THC	Beverage (1:100) %THC	Cookie (1:200) %THC	Gummy (1:250) %THC	Chocolate (1:300) %THC
0	0	0	0	0	0	0
500	2	6.25	0.005	0.01	0.0125	0.015
1000	4	12.5	0.01	0.02	0.025	0.03
2000	8	25	0.02	0.04	0.05	0.06
4000	16	50	0.04	0.08	0.1	0.12
8000	32	100	0.08	0.16	0.2	0.24

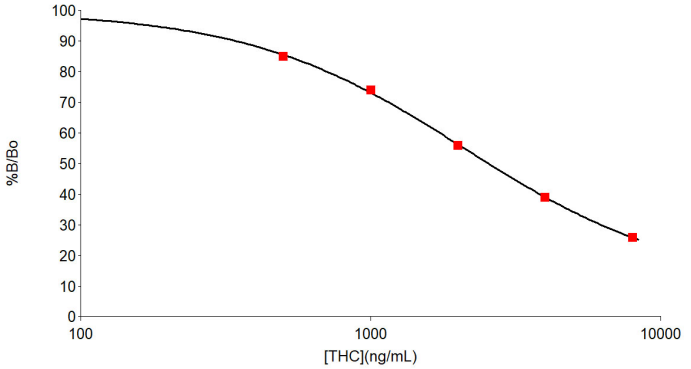
If a sample contains THC at a greater concentration than the highest standard, it should be diluted appropriately in 70% MeOH and re-tested. The extra dilution should be taken into account when expressing the final result.

Assay Characteristics

Intra-assay data from nine consecutive standard curves gave the following results.

D9-THC (ng/mL)	%B/B0	CV (%)
0	100	-
500	85	4.6
1000	74	5.4
2000	56	5.6
4000	39	5.1
8000	26	6.2

The graph below represents the data in the table above.



The assay also demonstrated very good intra-lot consistency with the following data based on 50 total runs with 3 kit lots over a period of 4 months with 2 different operators:

D9-THC (ng/mL)	%B/B0	CV (%)
0	100	-
500	85	2.3
1000	72	3.6
2000	55	4.6
4000	39	5.9
8000	26	6.5

Cross-Reactivity

The cross-reactivity of each analyte to the antibody was determined.

Analyte	Cross Reactivity (%)
D9-Tetrahydrocannabinol	100
D8-Tetrahydrocannabinol	107
Cannabinol	45
Tetrahydrocannabivarin	15
Cannabidiolic acid	<1.0
Tetrahydrocannabinolic acid A	<0.6
Cannabidiol	<0.3
Cannabidivarin	<0.3
Cannabigerol	<0.3
Cannabigerolic acid	<0.3
Cannabichromene	<0.3

Recovery

Samples were purchased from local dispensaries and tested. The % recovery was calculated by taking the THC measured in the sample divided by the THC content printed on the label multiplied by 100%. The recoveries are shown below and are based on 4 independent runs.

Product Name	Manufacturer	Sample Type	Mean % Recovery	%CV
Grape Pie*	Goochi	Flower	104	9.6
Strawberry Banana Sherbert*	Kaneh Gold	Flower	94	9.0
Jack Herer	Abx Absolute Extracts	Concentrate/ Vape	88	7.8
Strawberry Lemonade Slymer*	Moxie	Concentrate/ Badder	90	12.7
Strawberry Lemonade Drink	Cannabis Quencher	Edible/ Beverage	92	17.3
Surfer Cookie	Venice Cookie Co.	Edible/Cookie	89	12.8
Blenheim Apricot Gummy	Kiva Confections	Edible/Candy	85	5.0
Milk Chocolate Bar	Kiva Confections	Edible/Candy	101	18.3

*Sample was decarboxylated prior to testing.

Recovery Continued

Hemp samples were purchased from local markets and spiked with 0.3% THC. The % recovery was calculated by taking the THC measured in the sample divided by the known THC concentration spiked into the sample multiplied by 100%. The recoveries are shown below and are based on 4 independent runs.

Sample	Mean % Recovery	CV (%)
Hemp Oil	96	11
Hemp Seed	99	13
Hemp Oil Capsule	102	15
Hemp Gummy	113	9

Proficiency Testing

A pure solution of D9-THC was obtained from Emerald Scientific containing a concentration between 100-1000 µg/mL for individual laboratory testing. Our operator tested the blinded sample five independent times with the following results:

	Run1	Run2	Run3	Run4	Run5	Mean (µg/mL)	SD	%CV
Blind Sample	376.947	411.224	340.264	454.153	368.501	390.218	43.8	11.2

On average, the blind sample was 390.218 µg/mL, which was determined to have an assigned a value of 415 µg/mL with an acceptable range of 291-540 µg/mL. Our testing resulted in an acceptable value.

A proficiency testing sample of hemp oil containing an unspecified level of THC was also tested with the following results:

	Run1	Run2	Run3	Run4	Mean (µg/mL)	SD	%CV
Hemp oil with THC	0.8380	0.7595	0.6272	0.5708	0.6989	0.122	17.4

On average, the blind hemp oil proficiency testing sample was 0.6989 µg/mL, which had an assigned value of 0.874 µg/mL and acceptable range of 0.612-1.14 µg/mL.

Notes

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Innovation Based On Integrated Science

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