

## K2/Spice Synthetic Cannabinoids ELISA Assay Kit

### Catalog #4300-005 5 x 96 well plate

#### **Kit Description:**

The Tulip Biolabs K2/Spice Synthetic Cannabinoids ELISA Assay Kit is a 96-well microplate assay designed to test the presence of JWH-018, JWH-073, JWH-122, JWH-019, JWH-081, AM-2201 and related compounds and their metabolites mainly in urine samples. Using the positive control compound JWH-018 N-5-hydroxypentyl metabolite analog, the limit of detection in urine is 5ng/mL. Total assay time is approximately 2 hours. This assay does not cross-react significantly with JWH-250, UR-144, or XLR-11.

#### **Summary of the Procedure:**

The Tulip Biolabs K2/Spice Synthetic Cannabinoids ELISA Assay Kit is based upon the competitive binding of JWH-018 and other structurally related synthetic cannabinoids and their metabolites in the test sample and JWH-018-conjugate (JWH-018 conjugated to horseradish peroxidase) to the anti- JWH-018-coated 96-well microplate. In the procedure, 20µL aliquots of test samples (blanks, calibrators, quality control samples, and/or diluted unknown samples) are incubated with 100µL of JWH-018-conjugate in wells of a coated 96-well microplate. The wells are washed thoroughly with phosphate buffered saline, then TMB chromogenic substrate is added. A blue color is developed over 30 minutes at room temperature, then stopped by the addition of dilute acid. The final yellow intensity of the wells are measured at OD450nm, and the absorbance is inversely proportional to the concentration of JWH-018, related compounds and their metabolites in the sample. The limit of detection is approximately 5 ng/mL for JWH-018 N-5-hydroxypentyl analogue, a major JWH-018 metabolite in human urine. The JWH-018 N-pentanoic acid metabolite shows a similar response.

#### **Safety Warnings and Precautions:**

***This product is for research use only (RUO) and is not approved for therapeutic or diagnostic use.***

*All research reagents can be considered as being potentially hazardous. We therefore recommend that the contents of this kit be handled only by qualified laboratory personnel who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately.*

#### **Materials Provided:**

**NOTE ON STORAGE:** Store all components at 4°C. Place unused microwell strips back into the sealed pouch containing desiccant.

- JWH-018 ASSAY 96-WELL STRIPWELL PLATE, 5 PLATES (cat. #K301)
- JWH-018 CONJUGATE, 60mL (cat. #K302)
- TMB SUBSTRATE, 60mL (cat. #K203)
- STOP SOLUTION (0.2N HCl), 60mL (cat. #K204) **CAUTION: MILDLY CAUSTIC SOLUTION! AVOID CONTACT WITH SKIN AND EYES! WASH AFFECTED AREAS IMMEDIATELY WITH WATER!**
- PHOSPHATE BUFFERED SALINE, 2 packets sufficient to make 2 x 1L (cat. #K205)

#### **Additional Materials and Equipment Required but not Supplied:**

- Calibrator standards (user supplied or available from Tulip Biolabs Cat. #9023).
- Precision pipettes to deliver volumes of 20 and 100µL.
- Multi-tip or repeating pipettes to deliver 100µl and/or 325µL (optional).
- Automated microplate washer (optional) or absorbent paper (paper towels) for blotting.
- Microplate reader capable of reading OD450 nm.
- Phosphate buffered saline (20mM sodium phosphate, 150mM NaCl, pH 7.2-7.4) for sample dilution and microplate washing (only required if supplied packets are not of sufficient quantity).
- Microplate covers (e.g: Costar 3930 microplate cover)

#### **Kit Storage and Stability:**

The kit components are stable for at least 3 months from date of shipment when stored at 4°C (see lot specific expiration date). Place unused microwell strips back into the sealed pouch containing desiccant. Do not use components from different lot shipments. Note: Unopened phosphate buffered saline packets, #K205, are stable indefinitely and can be used with any kit lots.



**Specimen Storage:**

- Calibrator standards at -20 or -70°C (user supplied or available from Tulip Biolabs Cat. #9023).
- Specimen and quality control samples (e.g.: urine, plasma) at -20 or -70°C.

**Preparation of Experimental Samples:**

Prepare phosphate buffered saline solution, Cat. #K205, by completely dissolving each packet into 1 liter dH<sub>2</sub>O. Dilute test samples in phosphate buffered saline to minimize matrix effects, if required. Typically, human urine samples can be used directly. User must determine appropriate dilutions for test samples.

**Assay Procedure:**

*Note: During incubations, cover the microplate (e.g. Costar 3930 microplate cover).*

1. Allow all kit components to equilibrate to room temperature.
2. Dilute test specimens with phosphate buffered saline to minimize matrix effects, if required.
3. Add 20µL of test samples (blanks, calibrators, quality control samples, and test specimens) to wells of the microplate (#K301) as required by the user (see example plate set-up).
4. Wait 5 min, then add 100µL of JWH-018 conjugate (#K302) to each well. Tap each side of the plate holder to ensure thorough mixing.
5. Incubate plate for 60 minutes at room temperature (20-25°C) with gentle agitation.
6. Wash the plate wells 5-times with 325µL of phosphate buffered saline using a multi-tip pipette or 4-times with an automated plate washer. Be careful that the pipette tips do not touch the well bottoms.
7. After washing, invert and gently tap the plate twice onto absorbent paper placed on the benchtop to remove residual liquid (not required if using an automated microplate washer).
8. Add 100µl TMB SUBSTRATE (#K203) to each well. Tap each side of plate holder to ensure thorough mixing.
9. Incubate at room temp for 30 min with gentle agitation, preferably in the dark or dim light. The wells will develop colorless to blue in color.
10. Add 100µL STOP SOLUTION (#K204) to each well. **CAUTION: MILDLY CAUSTIC SOLUTION! AVOID CONTACT WITH SKIN AND EYES!** The wells will immediately change to a yellow color.
11. Read OD at λ450nm in a microplate reader between 5 to 30 min after STOP SOLUTION addition. Be careful to eliminate any air bubbles in the wells before reading. Note: Read OD at λ600nm and subtract from the OD450nm values well for well to adjust for light scattering (particulates, bubbles, etc), if desired.

NOTE: The above suggested assay procedure can be modified to better suit the users current laboratory equipment and set-up. In all circumstances, the user must validate any method for suitability of their intended purpose.

**Data Analysis:**

Calculate %OD/OD<sub>0</sub> from the OD450 (or OD450 – OD600 to account for light scattering) values as follows:

$$\%OD/OD_0 = 100 * OD450 / OD450_{blank}$$

where OD450 is the test sample value and OD450<sub>blank</sub> is the average blank value.

A cut-off value for %OD/OD<sub>0</sub> can be used to determine the presence of JWH-018 and/or other synthetic cannabinoids and their metabolites in test samples. Recommended standards are as follows (JWH-018 N-pentanoic acid metabolite can be substituted):

StdBlank: Buffer only (phosphate buffered saline).

StdCutoff: 5 ng/mL JWH-018 N-5-hydroxypentyl metabolite prepared in phosphate buffered saline.

QCpos: 100 ng/mL JWH-018 N-5-hydroxypentyl metabolite prepared in phosphate buffered saline.

In suspect samples, confirmation must be made using an alternative analytical method, for example LC-MS-MS.

**Limitation on use:**

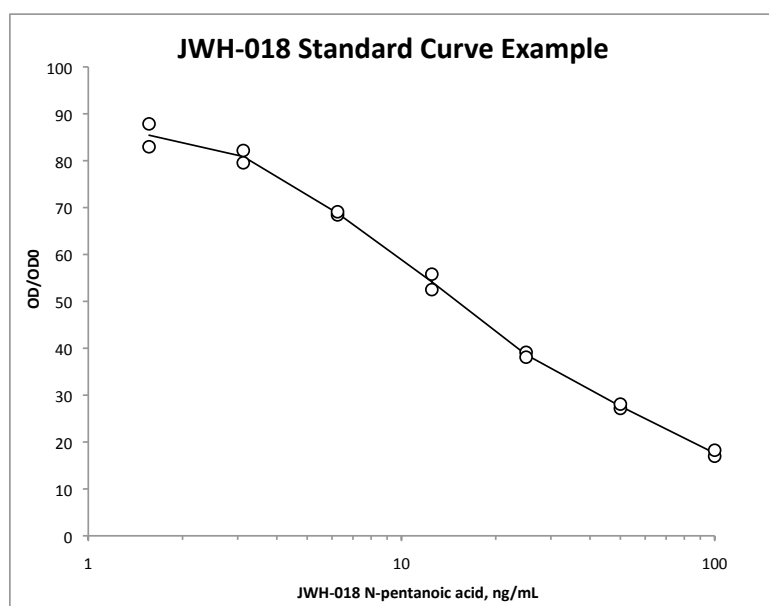
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### Drug and Metabolite Cross-Reactivity Relative to JWH-018-5-hydroxy metabolite

Compound	Cross-reactivity, %
JWH-122 4-hydroxy	200*
JWH-019 6-hydroxy hexyl	125*
JWH-073 N-4-hydroxy butyl	125*
JWH-081 O-desmethyl-4-hydroxy	125*
JWH-018 5-hydroxy	100
JWH-018 N-pentanoic	92.8
MAM-2201 4-hydroxy	86.5
JWH-250 N-pentanoic	32.1
JWH-250 4-hydroxy	24.6
PB-22 5-hydroxy	9.1
PB-22 N-pentanoic	8.6
UR-144 N-pentanoic	5.8
UR-144 5-hydroxy	5.2
AB-PINACA 5-hydroxy	neg
ADB-PINACA	neg
MN-25	neg
NNEI	neg
ADB-FUBINACA	neg
AKB48 N-pentanoic	neg
AKB48 4-hydroxy	neg
AB-CHMINACA M1A 4-hydroxy	neg
ADB-PINACA 4-hydroxy	neg
AB-FUBINACA M2 -COOH	neg
PX-1	neg

\* From A. Arntson *et al.* (2013) *J. Analyt. Toxicol.* **37** 284. See ref for additional cross-reactivity data.



#### Example Results in Human Urine:

Spice/K2 Incurred Urine Samples		
Sample No.	%OD/OD0	User?
1	109.9	ND
2	32.2	DETECTED
3	79.9	DETECTED
4	49.6	DETECTED
5	50.5	DETECTED
6	38.0	DETECTED
7	104.0	ND
8	100.4	ND
9	117.4	ND
10	31.6	DETECTED
11	7.7	DETECTED
12	77.5	DETECTED
StdBlank	100.0	-
StdCutoff	82.4	-
QCpos	42.8	-

StdBlank: Buffer only. StdCutoff: 20 ng/mL N-pentyl hydroxy metabolite.

QCpos: 100 ng/mL N-pentyl hydroxy metabolite.

Note: Urine samples 1, 7, 8, and 9 are neg control users.

