UR-144/XLR-11 (K2/Spice) Synthetic Cannabinoids ELISA Kit

Catalog #4500-005 5 x 96 well plate

Kit Description:
The Tulip Biolabs UR-144/XLR-11 (K2/Spice) Synthetic Cannabinoids ELISA Kit is a 96-well microplate assay designed to test the presence of UR-144, XLR-11 and its metabolites in samples such as urine, whole blood, serum, and plasma. Using UR-144 N-5-hydroxypentyl or N-pentanoic acid metabolite standards, the limit of detection in buffer is <0.5ng/mL. Total assay time is approximately 2 hours. This assay does not cross-react significantly with JWH-018 or JWH-250.

Summary of the Procedure:
The Tulip Biolabs UR-144/XLR-11 (K2/Spice) Synthetic Cannabinoids ELISA Kit is based upon the competitive binding of UR-144, XLR-11, and their metabolites in the test sample with the UR-144-conjugate (UR-144 conjugated to horseradish peroxidase) to the anti- UR-144-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 UR-144-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, and then stopped by the addition of dilute acid stop solution. The final yellow intensity of the wells is measured at OD450nm, and the absorbance is inversely proportional to the concentration of UR-144, XLR-11, and their metabolites in the sample. The assay is sensitive to approximately 1ng/mL for UR-144 N-5-hydroxypentyl or N-pentanoic acid analogues, the major UR-144/XLR-11 metabolites in human urine.

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.
- UR-144 ASSAY 96-WELL STRIPWELL PLATE, 5 PLATES (cat. #K501)
- UR-144 CONJUGATE, 60mL (cat. #K502)
- *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. #9025).*
- *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 absorbant 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).*

Kit Storage and Stability:
The kit components are stable for at least 3 months from date of shipment when stored at 4˚C. 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. #9025).*
- *Specimen and quality control samples (e.g.: urine, blood) 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_2O. Dilute test samples in phosphate buffered saline to minimize matrix effects, if required. User must determine appropriate dilutions for test samples.

***Assay Procedure**:*

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 (#K501) as required by the user (see example plate set-up).
4. Wait 5 min, then add 100µL of UR-144 conjugate (#K502) 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 plate 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.

***Data Analysis**:*

Calculate $\%OD/OD_0$ from the OD450 values as follows:

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

where OD450 is the average test sample value and $OD450_{blank}$ is the average blank value.

A cut-off value for $\%OD/OD_0$ can be used to determine the presence of UR-144, XLR-11 and their metabolites in test samples. Recommended standards are as follows, however the user must determine appropriate values:

StdBlank: Buffer only (phosphate buffered saline).
StdCutoff: 1 ng/mL N-5-hydroxypentyl or N-pentanoic acid metabolite prepared in phosphate buffered saline.
QCpos: 10 ng/mL N-5-hydroxypentyl or N-pentanoic acid metabolite prepared in phosphate buffered saline.

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

***Limitation on use**:*

This product is for research use only (RUO) and is not approved for therapeutic or diagnostic use. In all circumstances, the user must validate the method for suitability of their intended purpose.

Table 1. Drug and Metabolite Cross-Reactivity Relative to UR-144-5-OH (5 ng/mL)

COMPOUND	X-reactivity, %
UR-144-5-OH (calibrator)	*100*
UR-144 N-pentanoic acid	100
UR-144-4-OH	50
XLR-11 4-OH	50
XLR-11	8
UR-144	6
JWH-018 5-OH	neg
JWH-250 4-OH	neg
JWH-250 5-OH	neg
JWH-018	neg
AM2201	neg
Win 55-212-3	neg
RCS-4 Desmethyl-4-OH	neg
JWH-081 Desmethyl-4-OH	neg
JWH-081 Desmethyl-5-OH	neg
JWH-250 4-OH	neg
JWH-018 5-OH	neg
JWH-210 4-OH	neg
JWH-022 3-OH	neg
JWH-022 5-OH	neg
JWH-210 5-OH	neg
JWH-019 6-OH Hexyl	neg
JWH-073 3-OH Hexyl	neg
JWH-122 4-OH	neg
JWH-019 5-OH	neg
AM2201 4-OH	neg
JWH-073 4-OH	neg
JWH-018 4-OH	neg
JWH-122 5-OH	neg
JWH-018 Adamantyl	neg
JWH-018 5-Chloropentyl	neg
JWH-018 5-OH Glucuronide	neg
EDDP	neg
Methadone	neg
Methamphetamine	neg
Codeine	neg
Morphine	neg
PCP	neg
Cocaine	neg
Benzoylecgonine	neg
THC	neg
THC 11-OH	neg
THC Carboxy	neg
Cannabadiol	neg
MDMA	neg

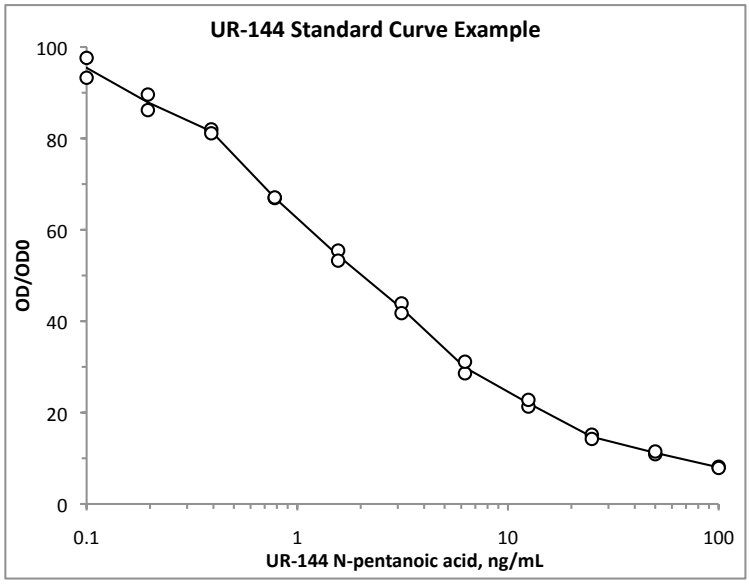

Figure 1. UR-144 N-pentanoic acid standard curve in phosphate buffered saline, example results.