PB-22 (QUPIC) Synthetic Cannabinoid ELISA Kit

Catalog #4600-005 5 x 96 well plate

Kit Description:
The Tulip Biolabs PB-22 (QUPIC) Synthetic Cannabinoid ELISA Kit is a 96-well microplate assay designed to test the presence of PB-22 metabolites mainly in urine samples. Using PB-22 N-pentanoic acid metabolite standard, the limit of detection in buffer is <0.25ng/mL. Total assay time is approximately 2 hours. This assay does not cross-react significantly with AKB48 or UR-144.

Summary of the Procedure:
The Tulip Biolabs PB-22 (QUPIC) Synthetic Cannabinoid ELISA Kit is based upon the competitive binding of PB-22 (QUPIC) metabolites in the test sample with a PB22-conjugate (PB-22 conjugated to horseradish peroxidase) to the anti-PB22-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 PB22-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 PB-22 metabolites in the sample. The assay is sensitive to approximately 0.25ng/mL for PB-22 N-5-hydroxypentyl or N-pentanoic acid analogues, the major PB-22 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.
- PB-22 ASSAY 96-WELL STRIPWELL PLATE, 5 PLATES (cat. #K601)
- PB-22 CONJUGATE, 60mL (cat. #K602)
- 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. #9026).
- 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).
- 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. 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. #9026).
- Specimen and quality control samples (e.g.: urine) 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:
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 (#K601) as required by the user (see example plate set-up).
4. Wait 5 minutes, then add 100µL of PB-22 conjugate (#K602) 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 2 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 substract from the OD450nm values well for well to adjust for light scattering (particulates, bubbles, etc), if desired.

Data Analysis:
Calculate $\%OD/OD_0$ 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 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 PB-22 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-pentanoic acid metabolite prepared in phosphate buffered saline.
QCpos: 10 ng/mL 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 PB-22 N-5-hydroxy metabolite (100 ng/mL)

Compound	Cross-reactivity, %
PB22 N-5-hydroxy	100.0
PB22 N-pentanoic acid	99.6
JWH250 N-pentanoic acid	53.3
JWH250 N-4-hydroxy	46.6
NNEI (MN-24)	23.1
JWH018 N-5-hydroxy	22.1
MAM2201 N-4-hydroxy	20.1
JWH018 N-pentanoic acid	13.8
ADB-FUBINACA	neg
MN25	neg
AB-PINACA N-5-hydroxy	neg
ADB-PINACA	neg
UR144 N-5-hydroxy	neg
UR144 N-pentanoic acid	neg
AKB48 N-4-hydroxy	neg
AKB48 N-pentanoic acid	neg

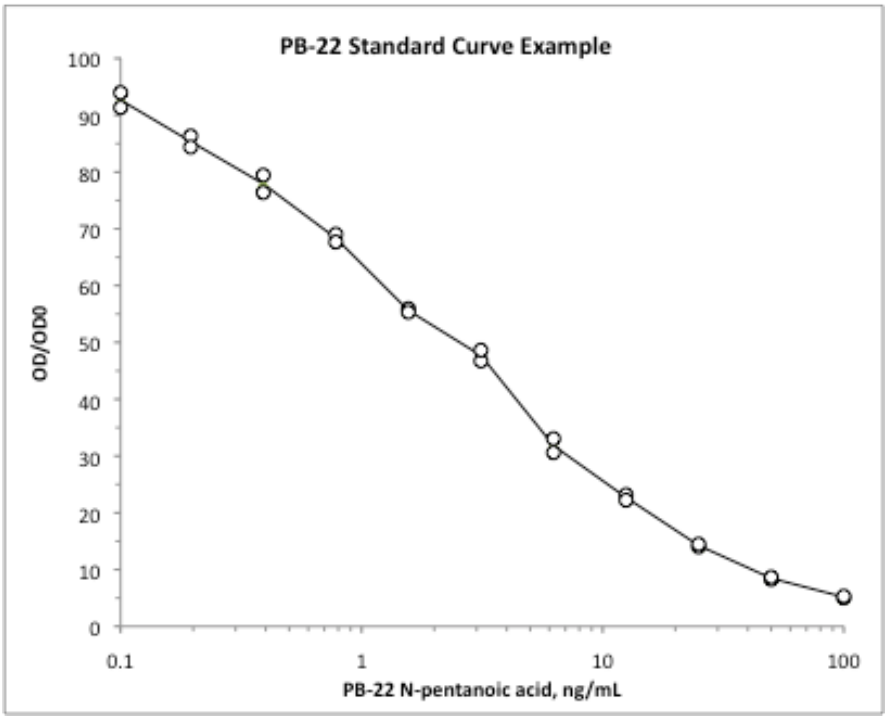

Figure 1. PB-22 N-pentanoic acid standard curve in phosphate buffered saline, example results.

Example of plate set-up (Note: The user must determine the appropriate layout for their procedure.):

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	StdBlank											
B	StdBlank											
C	StdBlank											
D	StdBlank											
E	StdCutoff											
F	StdCutoff											
G	QCpos											
H	QCpos											

Note: StdCutoff and QCpos may be made up in phosphate buffered saline, or in sample matrix diluted in phosphate buffered saline as determined by user.