PAH (*p*-Aminohippuric Acid) Colorimetric Assay Kit

(Catalog #K860-100; 100 assays; Store at -20°C)

I. Introduction:

p-Aminohippuric acid (PAH) is a derivative of hippuric acid and useful as a diagnostic agent for the measurement of renal plasma flow. About 20-30 % is completely filtered by the glomerulus and not readsorbed by the tubules. The remainder which bypasses the glomerulus and enters the tubules is completely secreted. At low doses, PAH is almost completely removed with one-pass through the kidneys. Hence, the venous concentration of PAH is close to zero and PAH has been used to determine the effective renal plasma flow (eRPF) from the plasma. The venous concentration is usually < 10 % that of the plasma concentration so eRPF slightly underestimates the actual RPF. This error is generally accepted because of the ease with which PAH infusion allows calculation of eRPF.

$$eRPF = ([PAH]_U / [PAH]_P) \text{ x Urine Volume}$$

BioVision's assay utilizes a 96-well format in which PAH reacts with Dimethylamino-cinnamaldehyde (DACA) which gives a strongly colored derivative at 550 nm. The amounts of PAH in urine and plasma can easily be quantified.

II. Kit Contents:

Component	K860-100	Cap Code	Part Number
TCA (15 %)	15 ml	NM	K860-100-1
DACA solution	15 ml	NM/Brown	K860-100-2
PAH Standard (10 mg/ml)	100 µl	Yellow	K860-100-3

III. Storage and Handling:

Store kit at -20°C, protect from light. Keep reagents on ice during use.

IV. Reagent Preparation:

All components are stable for at least 1 year from the date of shipment if kept at -20°C.

V. Assay Protocol:

1. **Standard Curve Preparation:** Dilute the PAH Standard to 0.1 mg/ml by adding 10 µl of the PAH Standard to 990 µl of distilled water. Take 200 µl of the 0.1 mg/ml Standard and add 600 µl of the 15 % TCA solution, mix. Add 0, 10, 20, 30, 40, 50 µl into a series of wells. Adjust volume to 50 µl/well with distilled water to generate 0, 0.25, 0.50, 0.75, 1.00, 1.25 µg/well of the PAH Standard.
2. **Sample Preparation:** Renal plasma flow determination requires both plasma and urine samples. For higher accuracy studies, in some cases, plasma samples bracket the urine sample collection time and interpolation determines the plasma PAH concentration that best corresponds to the time the urine sample was obtained.

   Plasma: Dilute 50 µl EDTA plasma or serum with 50 µl of the 15% TCA solution. Vortex and place on ice for 5 min. Centrifuge to pellet the precipitated protein. Use 50 µl of the clear supernatant per well.

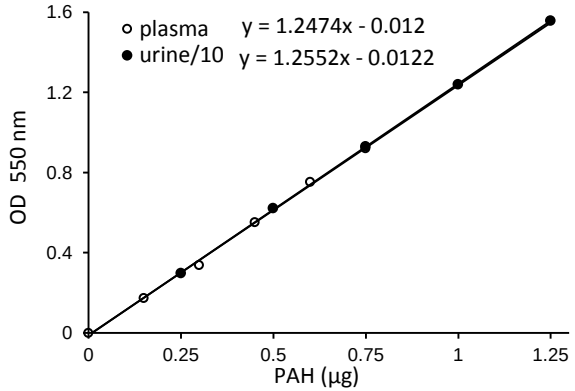
   Urine: Dilute 50 µl with 50 µl of the 15 % TCA solution. Vortex and place on ice for 10 min. Centrifuge to pellet any precipitated protein. Take 10 µl and dilute with 190 µl distilled water. Use 50 µl/well for testing.
3. **Reaction:** Add 150 µl of the DACA to each well containing Standard or test samples. Incubate the reaction for 30 min at room temperature, protect from light.
4. Measure absorbance at 550 nm in a microplate reader.
5. **Calculations:** Subtract the 0 Standard background reading from all readings. Plot the Standard Curve. Apply sample readings to the PAH Standard Curve. PAH concentration in samples can be calculated:

   Concentration = (A/V) * D = PAH (µg/µl)

   If sample volumes other than recommended in this protocol are used

   Where: **A** = Amount of PAH determined from the Standard Curve (in µg).
   D = Dilution factor (2 for plasma, 40 for urine)
   V = Volume of sample added per well in microliters (in µl).

NOTE: DACA can react with other aromatic amines and indoles. Common drugs such as sulfonamides and acetaminophen and their metabolites are included among those which can react. If the presence of these is expected or suspected, the chemical should be tested for reaction with DACA at the concentrations expected



References:

1. Agarwal, R., Am. J. Physiol Renal Physiol (2002) 283,F236-F241
2. Schwartz,L. B., Gewertz, B. L., Bissell M. G., Clin Chem (1988),165

RELATED PRODUCTS:

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Pyruvate Assay Kit	Glycogen Assay Kit
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Creatine Assay Kit	Fatty Acid Assay Kit
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Cholesterol Assay Kit	Urea Assay Kit
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