3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

PAH (*p*-Aminohippuric Acid) Assay Kit

Catalog Number **MAK101**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

p-Aminohippuric acid (PAH), a derivative of hippuric acid, is used in the measurement of renal plasma flow (RPF). Renal plasma flow measures the volume of plasma, which enters the kidney in a given period of time. To determine effective renal plasma flow, PAH is measured in a plasma sample (P_{PAH}) and a urine sample (U_{PAH}). The volume of urine flow (V) is also required. The equation to calculate renal plasma flow is the following:

$$RFP = [U_{PAH}/P_{PAH}] \times V$$

The PAH (*p*-Aminohippuric Acid) Assay kit can be used for measuring PAH in a plasma and urine samples. PAH reacts with 4-(dimethylamino)cinnamaldehyde (DACA) to generate a compound, which can be detected spectrophotometrically at 550 nm.

Components

The kit is sufficient for 100 assays in 96 well plates.

TCA, 15% Catalog Number MAK101A	15 mL
DACA Solution Catalog Number MAK101B	15 mL
PAH Standard, 10 mg/mL Catalog Number MAK101C	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Keep reagents on ice during use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

All components remain active for 2 months when stored at –20 °C.

Procedure

All samples and standards should be run in duplicate.

PAH Standards for Colorimetric Detection

Dilute 10 µL of the 10 mg/mL PAH Standard with 990 µL of water to prepare a 0.1 mg/mL solution. Take 200 µL of the 0.1 mg/mL standard and add 600 µL of the 15% TCA solution, mix well. Add 0, 10, 20, 30, 40, and 50 µL of the TCA/PAH standard solution directly into a 96 well plate, generating 0 (blank), 0.25, 0.5, 0.75, 1.0, and 1.25 µg/well standards. Add water to each well to bring the volume to 50 µL.

Sample Preparation
Renal plasma flow determination requires both plasma and urines samples.

Plasma: Dilute 50 µL of EDTA plasma or serum with 50 µL of 15% TCA solution. Mix well and incubate on ice for 5 minutes. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material. Add 50 µL of supernatant to wells.

Urine: Dilute 50 µL of urine with 50 µL of 15% TCA solution. Mix well and incubate on ice for 10 minutes. Centrifuge samples at 13,000 × *g* for 5 minutes to remove insoluble material. Take 10 µL of sample and dilute with 190 µL of water. Add 50 µL of diluted sample to wells.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

DACA can react with other aromatic amines and indoles in samples. Common drugs such as sulfonamides and acetaminophen, and their metabolites may react with DACA. If the presence of any of these compounds is expected or suspected, the chemical should be tested for reaction with DACA at the concentrations expected.

Assay Reaction
1. Add 150 µL of the DACA solution to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Cover the plate and protect from light during the incubation.

2. Measure the absorbance at 550 nm (A_{550}).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) PAH standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Note: A new standard curve must be set up each time the assay is run.

The amount of PAH present in the samples may be determined from the standard curve.

Concentration of PAH

$$C = S_a/S_v \times D \times 1{,}000$$

S_a = Amount of PAH in unknown sample from standard curve (µg)
S_v = Sample volume (50 µL)
D = dilution factor (2 for plasma, 40 for urine)
1,000 = conversion factor from µL to mL
C = Concentration of PAH in sample (µg/mL)

Sample Calculation
Amount of PAH in plasma (S_a) = 0.48 µg (from standard curve)
Sample volume (S_v) = 50 µL
Dilution factor = 2

Concentration of PAH in plasma sample

$$0.48\ \mu g/50\ \mu L \times 2 \times 1{,}000 = 19.2\ \mu g/mL$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of improperly stored reagents	Store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

SS,MF,LS,MAM 06/20-1