ISOQUANT® Isoaspartate Detection Kit

INSTRUCTIONS FOR USE OF PRODUCT MA1010.

Quick PROTOCOL

HPLC Detection Protocol

Reagent Preparation

1. Prepare the SAM stock solution (1:10) in NANOpure® water. Store on ice and in the dark.
2. Dilute Isoasp-DSIP to 7.5µM in sample buffer. Store on ice.
3. Transfer the test samples (75–375pmol/reaction) to duplicate tubes and bring the final volume of each tube to 10µl with sample buffer.
4. Dilute SAH to 7.5µM in water. Store on ice.
5. Prepare master mix.

Component	Volume per Reaction
NANOpure® water	10µl
Reaction 5X Buffer	10µl
SAM stock solution	10µl
PIMT (add last)	10µl

Reaction Protocol

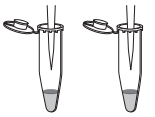
1. Assemble the blank (10µl sample buffer), reference standard (10µl Isoasp-DSIP) or the test samples (10µl for each sample).
2. Add 40µl of master mix to each tube and incubate at 30°C for 30 minutes.
3. Stop the reactions with 10µl of Stop Solution NR. Centrifuge for 8–10 minutes at 4°C. Store reactions in the dark at 4°C (–20°C for long-term storage).

Reverse Phase HPLC Analysis

1. Prepare SAH standard solution (10µl SAH standard, 7.5µM, in 50µl of NANOpure® water).
2. Transfer 55µl of each reaction to an autosampler vial and place in the autosampler tray.
3. Attach the Synergi™ Hydro-RP column to the HPLC instrument. Equilibrate the resin using the parameters: 10% mobile phase B, at 1ml/minute flow rate.
   Note: Always use a 40µl water blank as the first injection.
4. Inject 40µl of each reaction. Begin a gradient to 30% mobile phase B over 5 minutes, followed by a return to 10% mobile phase B over 30 seconds.
5. Hold the mobile phase B concentration at 10% for 7.5 minutes to prepare for the next sample.
6. For more than 25 samples: Wash the column after the 25th injection using the method described in the Technical Bulletin #TBI001. Inject 40µl of water and then proceed with the remaining samples.
7. Integrate the SAH peaks at 260nm and plot the standard curve in pmol versus peak area.

*See additional protocol information in Technical Bulletin #*TBI001*, available online at:*
www.promega.com

Test Preparation
Prepare and store the 0.1mM SAM solution, the 7.5µM IsoAsp-DSIP and the 7.5µM SAH solution on ice and in the dark.



Transfer test samples to duplicate tubes and bring to 10µl with sample buffer.

Prepare master mix.

Reaction Protocol
Add 40µl master mix to each reaction.

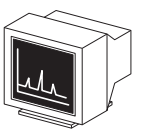
Incubate for 30 minutes at 30°C.

Add 10µl of Stop solution NR. Centrifuge for 5–7 minutes.

Reverse Phase HPLC Analysis
Prepare SAH standard sample.

Attach Synergi Hydro-RP Column to the HPLC. Equilibrate the resin with 10% mobile phase B.

Inject 40µl of the reaction onto the column.



Integrate the SAH peaks at 260nm and plot standard curve.

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Printed in USA. Revised 6/17
Part #9FB078