

PSR ¹⁸O Labeling Procedure

adapted from Larry's Lab 07/07/2007

Reagents

Trypsin Gold (Promega Cat # V5280)

DiH₂O

Neat Formic Acid (FA)

Porosyme Immobilized Trypsin beads (Applied Biosystems Cat # 2-3127-00)

Histidine solution (250 mM Histidine, pH 6.0 + 50 mM CaCl₂ for 10x buffer)

¹⁸O water

Acetonitrile (ACN)

0.45 μM filter (Millipore Cat # UFC30HV00)

Procedure

Follow Appropriate PSR Digestion Protocol until trypsin digestion step

Add Trypsin Gold in a 20:1 w/w ratio

Incubate overnight @ 37°C

Acidify each digest with 20 μL of neat formic acid

Perform Sep-pak clean up on each sample (refer to PSR Sep-pak cleanup procedure for instructions)

Take sample to dryness in a Speed Vac

* (3x) Take 20 μL of Poros solution and dilute to 100 μL in water, vortex, spin down beads, and pull off liquid*

Resuspend Poros beads in 60 μL of water

Add 8 μL of bead solution and 5 μL of Histidine solution to samples

Take sample to dryness in a Speed Vac

Add 45 μL of ¹⁸O water and 5 μL of ACN to one sample and 45 μL ¹⁶O and 5 μL of ACN to the other sample.

Vortex briefly to resuspend beads

Place in incubator/shaker overnight @ 37°C for ¹⁸O labeling

Filter out beads using 0.45 μM spin filters

Mix ^{16}O and ^{18}O samples together for mass spec analysis.

IMPORTANT NOTE: with a mixture of ^{16}O and ^{18}O water the labeling reaction will slowly reverse even without the trypsin present. This back exchange can render a labeling reaction useless in a matter of a few days. We usually save excess sample in 5 μL aliquots which are dried down and stored at -20°C to minimize this problem.