

# ***Pi*<sup>3</sup><sup>TM</sup> Methionine Reagent** **(*Pi*<sup>3</sup>-Met)**

## **Instruction Manual** **Revision 08.01.01**



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## Table of Contents

Introduction.....	1
Schematic.....	1
Chemistry.....	1
The Kit.....	2
Required Equipment.....	3
Required Material for One Isolation.....	3
Procedure for Use.....	4
A. Sample Preparation.....	4
B. $Pi^3$ -Met Pretreatment.....	4
C. Capture of Methionine Peptides.....	4
D. Wash after Capture.....	5
E. Methionine Peptide Release.....	5
F. Sample Collection.....	5
G. Sample Clean-Up.....	6
Appendix – Mass Spectrometry Search for Methionine-Containing Peptides.....	6
A. Search Strategy for Captured Methionine-Containing Peptides When Using MALDI and MS-Fit.....	6

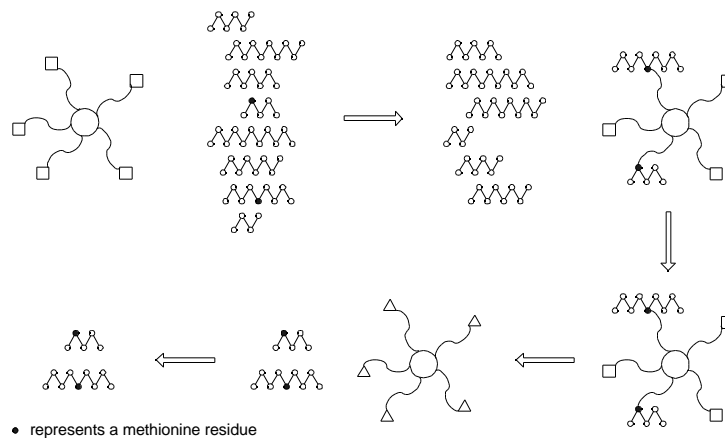
### General Suggestions for Maximizing Sample Recovery at Low Levels

- Rinse any 1.5 ml microcentrifuge tubes to be used, first with 1 ml 2% TFA and then with 1 ml of 70%  $CH_3CH$ /0.1% TFA.
- Use the same pipette tip for multiple transfers of the same sample.
- Use a 250  $\mu$ l pipette tip for sample transfer, instead of a 1 ml pipette tip.

## Introduction

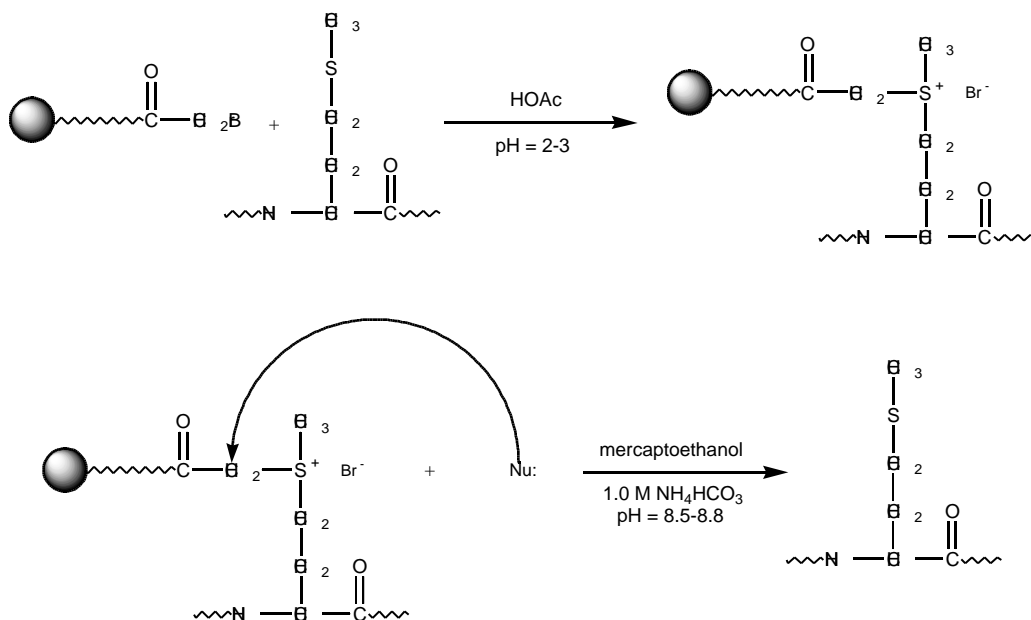
$Pi^3$  Methionine Reagent ( $Pi^3$ -Met) is a solid-phase reagent designed to isolate methionine-containing peptides from peptide mixtures. Methionine peptides are covalently bound to the  $Pi^3$ -Met, allowing for the removal of non-methionine peptides, and the subsequent release of the methionine peptides.

## Schematic



## Chemistry

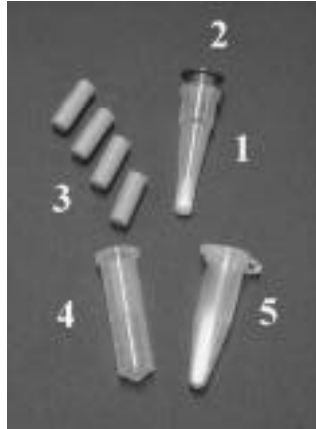
The  $Pi^3$ -Met solid-phase reagent is composed of derivatized porous glass beads designed to perform the following chemistry:



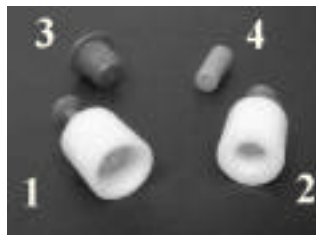
## The Kit

Each  $Pi^3$ -Met kit contains material enough for two isolation reactions. Included are:

- A. Two isolation packs, each comprising:
1. Spin tube containing  $Pi^3$ -Met (qty. 1)
  2. Red cap for spin tube top (qty. 1)
  3. Blue caps for spin tube bottom (qty. 4)
  4. Collection tube (qty. 1)
  5. Centrifuge tube containing reverse phase silica (50mg) (qty. 1).



- B. One accessory pack, comprising:
1. Top cap puncher (red handle) for red cap
  2. Bottom cap puncher (blue handle) for blue cap
  3. Extra red cap
  4. Extra blue cap.



Each isolation pack is designed to be used on a digestion of up to 5 nmol of protein.

## **Required Equipment**

- A. Vortex mixer with micro tube insert:
1. The holes in the insert must be, or must be modified to be, 3/8" (9mm) in diameter.
  2. If the holes in the insert are not through holes, the depth of the holes must be 1 3/4" (45mm) deep.
  3. We recommend the Vortex Genie II fitted with the 6" platform head and the 60 micro tube insert. This equipment is widely available from most laboratory suppliers.



- B. Bench top centrifuge:
1. Capable of 10000 RPM
  2. Capable of holding 1.5-2 ml microcentrifuge tubes.

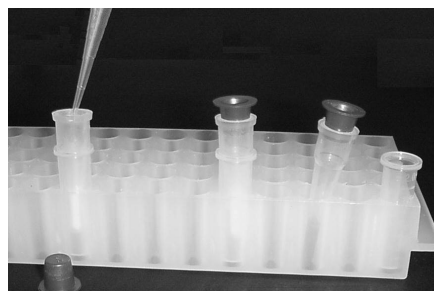


## **Required Material for One Isolation**

- A. Chemicals and solutions:
1. DI water (H<sub>2</sub>O) (~6 ml)
  2. Methanol (MeOH) (~3.5 ml)
  3. Glacial Acetic Acid (HOAc) (~30 µl)
  4. Mercaptoethanol (BME) (~10 µl)
  5. 1.0 M Ammonium Bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>), pH 9.0-9.1 (~150 µl)
  6. 10% HOAc in H<sub>2</sub>O (~1.5 ml)
  7. 2% Trifluoroacetic Acid (TFA) in water (~2 ml)
  8. 0.2 M BME in 25% HOAc (~1.5 ml)
  9. 20% Acetonitrile (CH<sub>3</sub>CN)/2% TFA in water (~75 µl)
  10. 70% CH<sub>3</sub>CN/0.1% TFA in water (~3.6 ml).

All chemicals used above are reagent grade or better.

- B. Other supplies:
1. Microcentrifuge tube rack (qty. 1)
  2. 1.5 ml microcentrifuge tube (qty. 2).



## Procedure for Use

### Notes:

- ◆ A microcentrifuge tube rack is used as a holder for the spin tube and the collection tube throughout this procedure.
- ◆ All liquid additions to the spin tube are done with the spin tube sitting in the collection tube.
- ◆ All vortexing steps are performed using the micro tube insert, except where noted.
- ◆ All centrifuge spins are done at 10000 RPM for 1 min.

### A. Sample Preparation

1. Bring the sample volume to 75  $\mu$ l with H<sub>2</sub>O. Mix well. Add 25  $\mu$ l HOAc. Mix well.

### B. $Pi^3$ -Met Pretreatment

1. Take the spin tube containing the  $Pi^3$ -Met and spin in the centrifuge to ensure that all material is settled at the bottom of the spin tube.
2. Remove the red cap from the spin tube. Using the top cap puncher, punch a hole in the cap to prevent pressure build-up in the tube.

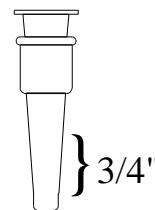
### Caution!

**Never vortex the spin tube without having the red cap firmly in place.**

3. Add 400  $\mu$ l of MeOH to the spin tube. Replace the red cap. Hold the collection tube together with the spin tube by hand, and vortex for 15 sec., adjusting the vortex speed until the liquid just reaches the red cap. Remove the red cap. Place the spin tube with the collection tube into a centrifuge and spin. Discard the liquid from the collection tube into a waste container.
4. Repeat Section B3 four (4) more times.
5. Pretreat the  $Pi^3$ -Met in the spin tube with 400  $\mu$ l of 10% HOAc per Section B3.
6. Repeat Section B5 two (2) more times.

### C. Capture of Methionine Peptides

1. Add the sample to the spin tube and replace the red cap. Insert the cap firmly.
2. Place a blue cap on the spin tube.
3. Place the spin tube in the vortex. Adjust the vortex speed to give a liquid level as shown in the drawing. Vortex for 4 hr.



#### **D. Wash after Capture**

1. Remove the spin tube from the vortex.
2. Remove the red cap and add 100  $\mu\text{l}$  of 0.2 M BME to the spin tube. Replace the red cap firmly and vortex for 30 min.
3. Remove the spin tube from the vortex.
4. Using the bottom cap puncher, punch a hole in the blue cap.
5. Remove the blue cap. Place the spin tube in the collection tube. Remove the red cap. Place the spin tube with the collection tube into a centrifuge. Spin. Remove the solution from the collection tube and save the solution if desired.
6. Repeat the procedure described in Section B3 above, but with the following solutions and volumes:
  - a) 3 times with 400  $\mu\text{l}$  of 0.2 M BME
  - b) 3 times with 400  $\mu\text{l}$  of 70%  $\text{CH}_3\text{CN}/0.1\%$  TFA
  - c) 3 times with 400  $\mu\text{l}$  of  $\text{H}_2\text{O}$ .

#### **E. Methionine Peptide Release**

1. Add 100  $\mu\text{l}$  of 1.0 M  $\text{NH}_4\text{HCO}_3$  and 7  $\mu\text{l}$  of BME to the spin tube. Place the red cap and then a new blue cap on the spin tube.
2. Vortex the spin tube for 3 hr. in a manner similar to Section C3 above.

#### **F. Sample Collection**

1. Rinse the collection tube with 1 ml of 70%  $\text{CH}_3\text{CN}/0.1\%$  TFA followed by 1 ml of  $\text{H}_2\text{O}$ .
2. Holding the spin tube upright, tap it gently three times on the bench. Using the bottom cap puncher, punch a hole in the blue cap and remove the blue cap carefully from the spin tube.
3. Place the spin tube into the rinsed collection tube. Remove the red cap carefully. Add any liquid remaining on the red cap to the spin tube. Spin.
4. Transfer the solution from the collection tube to a clean 1.5 ml microcentrifuge tube.
5. Add 50  $\mu\text{l}$  of 20%  $\text{CH}_3\text{CN}/2\%$  TFA to the spin tube. Place the red cap and a new blue cap on the spin tube.
6. Vortex the spin tube for 15 min. in a manner similar to Section C3 above.
7. Holding the spin tube upright, tap it gently three times on the bench. Using the bottom cap puncher, punch a hole in the blue cap and remove the blue cap carefully from the spin tube.
8. Place the spin tube into the collection tube. Remove the red cap carefully. Add any liquid remaining on the red cap to the spin tube. Spin.
9. Remove the solution from the collection tube and add to the solution already in the 1.5 ml microcentrifuge tube.
10. Add 300  $\mu\text{l}$  of 2% TFA to the spin tube and replace the red cap. Hold the collection tube with the spin tube by hand, and vortex for 15 sec., adjusting the vortex speed until the liquid just reaches the red cap.
11. Holding the spin tube/collection tube upright, tap them gently three times on the bench. Remove the red cap carefully. Add any liquid remaining on the red cap to the spin tube. Spin.

- Remove the solution from the collection tube and combine with the solution already in the 1.5 ml microcentrifuge tube.

### G. Sample Clean-Up

- Discard the used glass beads in the spin tube in an appropriate manner.
- Rinse the spin tube with H<sub>2</sub>O until no visible glass beads remain.
- Repeat the procedure described in Section B3 above, but with following solutions and volumes:
  - 2 times with 400  $\mu$ l of H<sub>2</sub>O
  - 2 times with 400  $\mu$ l of MeOH.
- Weigh and add an amount of silica, per the following table, to the spin tube.

Protein Sample Amount	Suggested Amount of Silica	Suggested Amount of Eluent
< 1 nmol	10 mg	30 $\mu$ l
1-2 nmol	20 mg	40 $\mu$ l
2-5 nmol	50 mg	50 $\mu$ l

Repeat the procedure described in Section B3 above.

- Add 400  $\mu$ l of 2% TFA to the spin tube. **DO NOT VORTEX!** Spin and discard the solution from the collection tube into a waste container.
- Add the sample solution in the 1.5 ml microcentrifuge tube from Section F12 to the spin tube. **DO NOT VORTEX!** Spin and discard the solution from the collection tube into a waste container.
- Repeat Section G5 two (2) times.
- Repeat Section F1.
- Add a volume of the eluent, 70% CH<sub>3</sub>CN/0.1% TFA per the table in Section G4. **DO NOT VORTEX!** Spin. Transfer the solution in the collection tube to a new 1.5 ml microcentrifuge tube.
- Repeat Section G9 two (2) times.
- Retain the sample in the 1.5 ml microcentrifuge tube for analysis.
- Dispose the waste material in an appropriate manner.

## Appendix – Mass Spectrometry Search for Methionine-Containing Peptides

### A. Search Strategy for Captured Methionine-Containing Peptides When Using MALDI and MS-Fit

- Creating Methionine/Methionine Oxidation Doublets:
  - For each peak in the mass table, create an artificial oxidation doublet by adding 15.99 Da. to each peak.
  - Search each doublet individually using the methionine oxidation option.
  - Require that there be two matches for each doublet.
  - Require that any correctly identified protein have at least one methionine moiety in every searched peptide.
  - Pool data from b)–d) above to determine protein(s).