Immobilized Trypsin

INSTRUCTIONS FOR USE OF PRODUCTS V9012 AND V9013

Quick PROTOCOL

1. Determine the reaction volume and resin volume required for digestion based on the information provided in Table 1.
2. Make protein digestion mixture (protein has been denatured, reduced and/or akylated):
   a. Add protein to a 1.5ml tube.
   b. Add acetonitrile to a 40% final concentration.
   c. Bring the digestion to the final reaction volume specified in Table 1 using 50mM ammonium bicarbonate.
   d. Vortex to mix.
3. Label each spin column. Remove the plug at the base of the column by twisting.
4. Place the columns into 1.5ml centrifuge tubes.
5. Determine the amount of resuspended resin to use (Table 1), resuspend the resin by gentle rocking, shaking or tapping, and pipet the resuspended resin into the spin column using a 1ml pipette. Centrifuge for 5 seconds to remove liquid from the resin.
6. Wash resin by adding the recommended amount of 50mM ammonium bicarbonate to each spin column (Table 1). Centrifuge for 5 seconds.
7. Remove liquid from the tube.
8. Repeat Steps 6 and 7 two more times for a total of three washes.
9. Centrifuge for 5 seconds to remove any excess liquid.
10. Remove the spin columns, and place them into clean, labeled 1.5ml tubes.
11. Add the protein digestion solution directly to the resin in the spin column. If a droplet remains on the pipette tip, gently lower it to the resin.
12. Cap the tube loosely.
13. Incubate at room temperature for 30 minutes on the bench top.
14. Add appropriate amount of Peptide Recovery Buffer (Table 1) to the spin column. **Note:** You can centrifuge quickly without the Peptide Recovery Buffer; however, the recovery amount will be reduced.
15. Centrifuge quickly (~5 seconds) to remove the peptide solution from the resin.
16. Leave the peptide solution in the tube.
17. Repeat Steps 14 and 15. When using resin volumes >400µl, a second 1.5ml tube is needed for the second elution.
18. Remove the spin column, and save the peptide solution for analysis. **Note:** The recovered peptide solution contains 40% acetonitrile, which may require removal by lyophilization before analysis.

Table 1. Volumes Required for Protocol.

Amount of Protein to Be Digested	Digestion Mix Volume	Resuspended Resin Volume	Wash Buffer Volume	Peptide Recovery Buffer Volume
20–49µg	20µl	100µl	100µl	50µl
50–149µg	40µl	200µl	200µl	100µl
150–299µg	80µl	400µl	400µl	200µl
300–500µg	120µl	600µl	400µl	300µl

Additional information is available in the *Immobilized Trypsin Technical Manual* #TM077, available at: **www.promega.com/tbs**

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