

# Solution Preparation

**ATTENTION: All solutions need to be made FRESH right before the digestion. High purity reagents preferred (99+%).**

Eppendorf tubes must be siliconized. One suggestion: Polypropylene Eppendorf Tube, Sarstadt 72.690 & 72.699, Tel: 1-800-257-5101.

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## **50% ACN in dd H<sub>2</sub>O**

250 µl Acetonitrile + 250 µl doubly deionized H<sub>2</sub>O

## **100 mM Ambic**

100 µl of 1M Ammonium Bicarbonate (0.79 mg in 10 ml dd H<sub>2</sub>O) in  
900 µl of H<sub>2</sub>O

## **25 mM Ambic**

25 µl 1M Ambic + 975 µl dd H<sub>2</sub>O

## **10 mM DTT in 100 mM Ambic**

5 µl 2M Dithiothreitol (0.15 mg in 500 µl dd H<sub>2</sub>O) + 895 µl dd H<sub>2</sub>O +  
100 µl 1M Ambic

## **55 mM IAA in 100 mM Ambic**

10 mg Iodoacetamide + 100 µl 1M Ambic + 900 µl dd H<sub>2</sub>O

## **Trypsin solution**

5 µl 1M CaCl<sub>2</sub> + 50 µl 1M Ambic + 945 µl dd H<sub>2</sub>O + 125 µl 0.1 µg/µl  
trypsin in dd H<sub>2</sub>O

Must be sequencing grade trypsin. One suggestion: B-Mannheim  
sequencing grade, Modified Trypsin (highly purified), pH 7.5~9,  
Catalogue 1418033

## **Buffer solution without trypsin**

5 µl 1M CaCl<sub>2</sub> + 50 µl 1M Ambic + 945 µl dd H<sub>2</sub>O

**10 mM DTT in dd H<sub>2</sub>O**

5  $\mu$ l 2M DTT + 995  $\mu$ l dd H<sub>2</sub>O

**5% Formic Acid**

57  $\mu$ l stock Formic Acid (~88%) + 943  $\mu$ l dd H<sub>2</sub>O

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**Stock Solutions****1M Ambic**

0.79 g Ammonium Bicarbonate in 10 ml dd H<sub>2</sub>O

**2M DTT**

0.15 g DTT in 500  $\mu$ l dd H<sub>2</sub>O

**1M CaCl<sub>2</sub>**

0.15 g CaCl<sub>2</sub>·2H<sub>2</sub>O in 1ml dd H<sub>2</sub>O

**30 mM Potassium Ferricyanide**

10 mg Potassium Ferricyanide in 1 ml dd H<sub>2</sub>O

**100 mM Sodium Thiosulfate**

25 mg Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in 1 ml dd H<sub>2</sub>O