

## In-gel Digestion Material for MS Analysis

### Check!

- \_\_\_\_\_ 1. Polyacrylamide gel containing protein band (1~2 mm OD in size)
- \_\_\_\_\_ 2. 0.5 ml tubes (Eppendoff, Sarsteck and Axygen are recommended)
- \_\_\_\_\_ 3. pipetmen: p2, p10, p200, p1000
- \_\_\_\_\_ 4. Tips: 10 µl Tip, 200 µl Tip, 1000 µl Tip
- \_\_\_\_\_ 5. Powder free gloves
- \_\_\_\_\_ 6. Acetonitrile (ACN, HPLC grade, or better)
- \_\_\_\_\_ 7. Trifluoroacetic acid (TFA, HPLC grade, or better)
- \_\_\_\_\_ 8. Ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>)
- \_\_\_\_\_ 9. Potassium ferricyanide (for silver destain)
- \_\_\_\_\_ 10. Sodium thiosulfate (for silver destain)
- \_\_\_\_\_ 11. Dithiothreol (DTT)
- \_\_\_\_\_ 12. Iodoacetamide (IAA)
- \_\_\_\_\_ 13. Vacuum centrifuge (Speed-Vac)
- \_\_\_\_\_ 14. 56°C and 37°C water bath or oven
- \_\_\_\_\_ 15. Sonication bath
- \_\_\_\_\_ 16. Trypsin (sequencing grade, Promega)  
 Promega trypsin (Cat. #V5111) is sold in freeze dried form, 20 µg/vial.  
 The solid powder is solved in 1 mM hydrochloride acid (for Roche Diagnostic, Mannheim trypsin) or 50 mM acetic acid (for Promega trypsin) giving a concentration of 100 ng/µl, store 10 µl aliquots at -20 °C.
- \_\_\_\_\_ 17. Matrix: CHCA (α-cyano-4-hydroxycinammic acid)

### Solution:

- \_\_\_\_\_ 1. Ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>), pH 8.0:  
 10 mM (7.92 mg/10 ml)/50% (v/v) ACN  
 10 mM (7.92 mg/10 ml) in Milli Q water  
 20 mM (15.84 mg/10 ml) in Milli Q water  
 25 mM Na<sub>2</sub>CO<sub>3</sub>, pH 10 for Ruby stained sample destaining.
- \_\_\_\_\_ 2. Silver destain solution:  
 0.1 g Potassium ferricyanide, 0.15 g Sodium thiosulfate / 10 ml Milli Q water  
 or 0.1% H<sub>2</sub>O<sub>2</sub> for silver stain sample destaining.
- \_\_\_\_\_ 3. Reduction solution: 10 mM Dithiothreol (DTT, 1.54 mg/ml) in 10 mM NH<sub>4</sub>HCO<sub>3</sub>
- \_\_\_\_\_ 4. Alkylation solution: 55 mM iodoacetamide (IAA, 10.2 mg/ml) in 10 mM NH<sub>4</sub>HCO<sub>3</sub>
- \_\_\_\_\_ 5. 50% ACN, 1% TFA
- \_\_\_\_\_ 6. Trypsin solution: 20 ng/µl trypsin in 10 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8
- \_\_\_\_\_ 7. Matrix solution:  
 10 mg/ml in 50% ACN / 0.1% TFA – for Regular target plate  
 1 mg/ml in 50% ACN / 0.1% TFA – for AnchorChip target plate