In-gel Trypsin Digestion and Peptide Extraction

1. Digest protein.
    a. Prepare 20 μg/mL trypsin in 20 mM ammonium bicarbonate. Use only sequencing grade trypsin, which can be purchased from Sigma or Promega. We purchase Trypsin Gold from Promega (cat. no. V5280).

        i. Prepare a 1 mg/mL stock of trypsin in acetic acid buffer. For Promega Trypsin Gold, resuspend 100 μg lyophilized trypsin in 100 μL acetic acid buffer.
        ii. Aliquot 10 μL to 10 microfuge tubes and store at -80^{o}C.
        iii. Dilute each 10 uL aliquot with 500 μL of 20 mM ammonium bicarbonate.

            15.8 mg ammonium bicarbonate
            10 mL H_2O

    b. Add 10 μL trypsin solution to each gel plug (for 2.0 mm diameter gel plugs). Seal plate/tube. If working with gel bands, increase volume relative to gel amount.

    c. Incubate gel plugs at 37^{o}C for 2 hours-overnight.

2. Extract and dry peptides.
    a. Add 60 μL 50% acetonitrile/0.1% TFA to each gel plug (for 2.0 mm diameter gel plugs). Incubate at room temperature for 20 minutes. If working with gel bands, increase volume relative to gel amount.

        500 μL acetonitrile
        500 μL H_2O
        1 μL TFA

    b. Remove extract solution and save.

    c. Add 40 μL 50% acetonitrile/0.1% TFA to each gel plug (for 2.0 mm diameter gel plugs). Incubate at room temperature for 20 minutes. If working with gel bands, increase volume relative to gel amount.

    d. Remove extract solution and combine with earlier saved extract solution.

    e. Dry combined extract solution. Sample can be shipped overnight at ambient temperature.