Reduction and Alkylation Prior to In-gel Trypsin Digestion

1. Reduce disulfide bonds.
    a. Prepare 10 mM dithiothreitol (DTT) in 20 mM ammonium bicarbonate. For a cysteine-rich protein, use 50 mM DTT (7.7 mg DTT/mL).

        1.54 mg DTT
        1.58 mg ammonium bicarbonate
        1 mL H_2O

    b. Add 40 µL DTT solution to each gel plug (2 mm diameter) and incubate at one of the following temperatures. If working with gel bands, increase volume relative to gel amount.

        37°C for 1-2 hours
        56°C for 45 min
        60°C for 20 min

2. Alkylate cysteine residues.
    a. Prepare 100 mM iodoacetamide in 20 mM ammonium bicarbonate. For a cysteine-rich protein, use 200 mM iodoacetamide (37 mg iodoacetamide/mL).

        18.5 mg iodoacetamide
        1.58 mg ammonium bicarbonate
        1 mL H_2O

    b. Remove DTT solution and immediately add 40 µL iodoacetamide solution to each gel plug (2 mm diameter) and incubate at room temperature for 30 min in the dark. If working with gel bands, increase volume relative to gel amount.

3. Wash/dehydrate/dry gel plugs.
    a. Incubate 2.0 mm gel plugs with 100 µL 50 mM ammonium bicarbonate in 50 % methanol for 20 minutes at room temperature. If working with gel bands, increase volume relative to gel amount.

        79 mg ammonium bicarbonate
        10 mL methanol
        10 mL H_2O

    b. Remove methanol solution.

    c. Incubate 2.0 mm gel plugs with 100 µL 75 % acetonitrile for 20 minutes at room temperature. If working with gel bands, increase volume relative to gel amount.

        15 mL acetonitrile
        5 mL H_2O

    d. Remove acetonitrile solution. Dry gel plugs at 40 °C for 15-20 minutes. If working with gel bands, increase drying time to ensure that gel is completely dehydrated and "crisp".