In gel protein processing

This document describes a protocol for SDS-PAGE separation of a protein sample, extraction, digestion, and mass spectrometry analysis for identification. Although the protocol mainly describes extraction of a single protein band, it could also be used for extraction and preparation of multiple bands from a single, or multiple, lanes. This protocol starts after the gel has been run and in theory should be compatible with virtually any SDS-PAGE gel.

Quick links

Reagents and materials

- 1.5mL or 2.0mL Safe-Lock tubes (Thermo Scientific, CAT#05-402-25 or CAT#05-402-7)
- Acetonitrile, HPLC grade (Thermo Scientific, CAT#51101)
- Water, HPLC grade (Thermo Scientific, CAT#51140)
- Methanol, HPLC grade (Sigma, CAT#34860-4L)
- Acetic acid (Sigma, CAT#A6283)
- Brilliant blue (Sigma, CAT#B7920)
- Ammonium bicarbonate (Sigma, CAT#A6141-500G)
- TCEP (Sigma, CAT#580560)
- Chloroacetamide (Sigma, CAT#C0267)
- Urea (Sigma, CAT#U5128)
- Trypsin/rLysC mix (Promega, CAT#V5073)
- Calcium chloride (Sigma, CAT#C4901)
- Formic acid, HPLC grade (CAT#85178, Thermo Scientific)
- Thermomixer capable of holding 1.5mL or 2.0mL tubes (Eppendorf)
- Benchtop centrifuge (capable of holding 1.5mL or 2mL microfuge tubes)
- Sonicating water bath (optional)
- Razor blade (or some sort of clean cutting tool)
- Small, clean glass plate (this is useful for cutting the gel on)

Solution recipes

- Gel fixing solution (recipe for 50mL)
    - Methanol - 25mL
    - Water - 20mL
    - Acetic acid - 5mL
- Gel destaining solution (recipe for 50mL)
    - Methanol - 22.5mL

    - Water - 22.5mL
    - Acetic acid - 5mL
- Coomassie concentrated solution (only need this if you are staining your gel)
    - Methanol - 30mL
    - Acetic acid - 6mL
    - Brilliant blue - 1.2g
- Gel staining solution (recipe for 50mL) (only need this if you are staining your gel)
    - Methanol - 250mL
    - Water - 200mL
    - Acetic acid - 50mL
- Gel piece destaining solution (make 30mL)
    - 100mM ammonium bicarbonate - 237mg
    - 20% (v/v) acetonitrile - 6mL of 100% acetonitrile solution
    - Water - 24mL
- TC solution (need 40uL per sample, recipe for 1000uL)
    - 12.4mg TCEP
    - 18.6mg of CAA
    - water to 1000uL
- 100mM ammonium bicarbonate - 7.9mg for every 1mL of water
- 50mM acetic acid - 28.7uL of 17.4M acetic acid in 9.97mL of water
- 1M calcium chloride - 1.1g in 10mL of water
- Digestion solution
    - 10uL of 1M calcium chloride solution
    - 990uL of 100mM ammonium bicarbonate
- 2M urea (12mg for every 100uL of 100mM ammonium bicarbonate, prepare just before use) (need 100uL per sample)
- 1% formic acid - 100uL of formic acid in 10mL of water

Protocol

This protocol generally takes 2-3 days. I tried to break it up into major sections based on this. If you have already stained your gel, you can skip the staining steps. If you don't have a sonicating bath you can just vortex the solution in the steps below that call for it.

1. For staining of the gel:
    1. Remove gel from the running cassette and place carefully in a container with gel fixing solution (enough to cover the gel).
    2. Incubate the gel in fixing solution on a rotary mixer for 30-minutes and discard the fixing solution afterwards.
    3. Add gel staining solution (enough to cover the gel) and stain the gel for 30-minutes and discard the staining solution afterwards.
    4. Add gel destaining solution and shake until no visible background remains (2-3 hours). The destaining can be accelerated by changing the destain liquid frequently, or adding a folded up KimWipe to the container.
    5. Keep the gel in fixing solution for long term storage at this stage if desired.
2. To prepare gel bands for extraction and perform digestion:

1. Prepare a sufficient number of 2mL tubes for the number of gel bands you would like to cut out and add 200uL of the gel piece destain solution to each.
2. Place the gel on cutting surface (such as a glass plate) and cut out your band of interest using a clean razor blade.
3. Cut the extracted gel piece into small cubes (~1mm) and insert into the tube with gel piece destain solution in it.
4. Destain the gel pieces until no blue remains! This process can be accelerated by changing the gel piece destain solution frequently.
5. Remove and discard the destain and add 500uL of acetonitrile. Mix well by vortexing.
6. Once the gel pieces have turned completely white, discard the acetonitrile.
7. Add 100uL of TC solution (or enough to cover the gel pieces) and incubate for 30-minutes at room temperature (+21C).
8. Remove and discard any excess liquid and add 500uL of acetonitrile. Mix by vortexing.
9. Incubate until the gel pieces are completely white.
10. Discard the acetonitrile and add 200uL of 100mM ammonium bicarbonate and mix until the gel pieces are clear and hydrated again.
11. Discard the excess ammonium bicarbonate solution from the tubes and add 500uL of acetonitrile and mix until the gel pieces are completely white.
12. During the above incubation, prepare your trypsin mixture:
13. Reconstitute urea powder with an appropriate amount of Digestion solution. Vortex mix. NOTE - urea will increase the volume of the solution generally by the amount of powder you have weighed out. For example, to 120mg of urea, I would add 880uL of Digestion solution to make 1mL of solution.
14. Reconstitute a 20ug trypsin/rLysC vial using 100uL of 50mM acetic acid.
15. Transfer 1uL of trypsin per sample to a fresh tube, and add 100uL of prepared urea in Digestion solution per sample. Vortex mix and keep on ice.
16. Discard the excess acetonitrile.
17. Add 100uL of the trypsin mixture to the tubes (or enough to cover the gel pieces) and incubate for 2-hours at +30C in a Thermomixer with shaking at 800rpm.
18. Add 300uL of Digestion solution (no urea) to the gel pieces and incubate overnight at +30C in a Thermomixer with mixing at 800rpm.

3. To extract the generated peptides from the gel pieces:
    1. Spin digest tubes for 1-minute at 10,000g and transfer the supernatant liquid to a fresh labeled 1.5mL tube for each sample.
    2. Add 100uL of 1% formic acid to the tubes and sonicate in a water bath for 5-minutes.
    3. Spin the tubes for 1-minute at 10,000g and transfer the liquid to the 1.5mL tubes from Step 3.1.
    4. Repeat the previous two steps one addition time for a total of 2 extractions with 1% formic acid.
    5. Add 200uL of acetonitrile to the tubes and sonicate in a water bath for 5-minutes.
    6. Spin the tubes for 1-minute at 10,000g and transfer the liquid to the tubes containing the other material for the sample.
    7. Concentrate the peptide sample by evaporation. You can use a SpeedVac or a Lyophilizer for this purpose. The evaporated samples can be stored at -80C indefinitely.
4. The resulting samples should be desalted using SPE prior to analysis on the MS.