Partial trypsin digestion of cornified envelope proteins using ProteaseMax Surfactant (Promega)

ProteaseMAX™ Surfactant Digestion Protocols
Materials to Be Supplied by the User
Note: Mass spectrometry analysis is highly sensitive to contaminants. Use only the highest quality reagents and solvents (e.g., “mass spectrometry grade”).
• NANOpure® (or equivalent grade) water
• methanol
• ethanol
• 50mM NH_4HCO_3
• acetonitrile (ACN)
• 1M dithiothreitol (DTT)
• 0.55M iodoacetamide (IAA)
• trifluoroacetic acid (TFA)
• trypsin (We recommend Trypsin Gold, Mass Spectrometry Grade, Promega Cat.# V5280.)
• 50mM acetic acid

The following reagents are also required for in-solution digestions.
• –20°C acetone
• 8M urea

The following conditions are for a pilot experiment in which trypsin concentration and digestion time are varied.

In-Solution Digestion Protocol (sample protocol for membrane protein)
Be sure to use the correct concentration of ProteaseMAX™ Surfactant in the digestion step (0.05% for membrane proteins). Higher concentrations of surfactant might cause peptide loss or interfere with cleanup. This protocol was developed using 50µg of membrane protein extract from mouse heart.
Adjust volumes and quantity of reagents accordingly for larger amounts of protein. If urea is needed as a solubilizer, it can be used along with ProteaseMAX™ Surfactant. Use of a urea/ProteaseMAX™ Surfactant mix does not require any changes to the protocol.
Summary tables of volumes used are provided in Tables 1 and 2 at the end of the protocol. ProteaseMAX™ Surfactant concentrations given here refer to the concentration in the digestion reaction. You may use higher concentrations to solubilize protein.

Prepare CEs as indicated in protocol. For 1 pilot experiment, pool CEs from 4 embryos and resuspend in 200 µl of 2 % SDS, 50 mM NH_4HCO_3.

1. Precipitate CEs (50µg) from 4 neonatal embryos with 4 volumes of –20°C acetone at –80°C for 20 minutes. Collect the protein by centrifugation at 20.000 × *g* for 10 minutes at 4°C. Discard the supernatant, and rinse the pellet with 300µl of (–20°C) acetone. Air-dry the pellet for 3–5 minutes.
2. Solubilize protein as follows:
Solubilization with ProteaseMAX™ Surfactant/urea mix: Add 300 µl of 8M urea (dissolved in NANOpure® water), then add 400µl of 0.2% ProteaseMAX™ Surfactant/50mM NH_4HCO_3. Mix by vortexing or shaking on an orbital shaker as above.
3. Add 50mM NH_4HCO_3 to a final volume of 1.870 µl (= 93.5 µl/sample).
4. Add 20 µl of 0.5M DTT. Incubate at 56°C for 20 minutes. This sample is for 20 trypsin digestions: at the end of digestion, divide into 94.5 µl/digest.

5. Add 2.7µl of 0.55M iodoacetamide per sample. Incubate at room temperature in the dark for 15 minutes.
6. While performing Steps 4 and 5, dissolve trypsin to 1µg/µl with 50mM acetic acid and store on ice.
7. Add 1µl of 1% ProteaseMAX™ Surfactant and 0.2, 0.5, 0.75, 1 and 1.8µl of 1µg/µl trypsin. Incubate at 37°C for 0, 15, 30, 180 min.
8. Collect the condensate from tube walls by centrifugation at 20.000 × g for 10 seconds. Stop reaction by addition of 10 µl of 20 % SDS/20 mM DTT. Instantly heat 2 min at 95°C to stop trypsin activity. Immediately precipitate with 4 volumes of –20°C acetone at –80°C for 20 minutes. Collect the protein by centrifugation at 20.000 × *g* for 10 minutes at 4°C. Discard the supernatant, and rinse the pellet with 300µl of (–20°C) acetone. Air-dry the pellet for 3–5 minutes. Resuspend in 15 µl 0.1 % SDS, add 5 µl Laemmli sample buffer. Load sample on 15 % gel with 2.5 cm long stacking gel.

Pipetting scheme:

Table 1. Summary of In-Solution Solubilization/Digestion Reaction Volumes for Membrane Proteins.

Component	Membrane Protein (ProteaseMAX™ Surfactant only for solubilization) (µl)	Membrane Protein (ProteaseMAX™ Surfactant and urea for solubilization) (µl)
0.2% ProteaseMAX™ Surfactant:50mM NH_4HCO_3 (for solubilization)	20	20
8M urea	–	15
50mM NH_4HCO_3	73.5	58.5
0.5M DTT	1.0	1.0
0.55M iodoacetamide	2.7	2.7
trypsin (1µg/µl)	1.8	1.8
1% ProteaseMAX™ Surfactant (for digestion)	1.0	1.0
Final Volume	**100**	**100**

6. Appendix
6.A. Composition of Buffers and Solutions
6.B. Related Products
Product Size Cat. numbers
Trypsin Gold, Mass Spectrometry Grade 100µg V5280
Sequencing Grade Modified Trypsin* 100µg V5111
Sequencing Grade Modified Trypsin, Frozen* 100µg V5113
*For Laboratory Use.
50mM ammonium bicarbonate buffer
(pH~7.8)
Dissolve 98.8mg of NH_4HCO_3 in 25ml of NANOpure® water. Prepare the buffer immediately before use. Keep it at room temperature until use.
1M DTT
Dissolve 154.25mg of DTT in a final volume of 1ml of NANOpure® water. Store on ice.
25mM DTT

Dilute the 1M DTT stock solution 40-fold with 50mM NH_4HCO_3 immediately before use.

0.55M iodoacetamide

Dissolve 40.7mg of iodoacetamide in 400µl of 50mM NH_4HCO_3. Prepare immediately before use, and store in the dark.

iodoacetamide solution

Dilute 0.55M iodoacetamide solution 10-fold with 50mM NH_4HCO_3 immediately before use, and store in the dark.

8M urea

Dissolve 480.5mg of mass spectrometry-grade urea crystals in a final volume of 1ml NANOpure® water.