PROTEASEMAX IN-SOLUTION DIGESTION PROTOCOL (30 µg)

Buffers: 8 M Urea → 0.24 g, 300 µl HPLC H_2O

1 M NH_4HCO_3 (Ambic) → 0.079 g, 1 ml HPLC H_2O

50 mM NH_4HCO_3 (Ambic)→ 50 µl 1M NH_4HCO_3, 950 µl HPLC H_2O

0.5 M DTT → 0.03g, 400 µl 50 mM Ambic

0.55 M IAA →0.05 g, 500 µL 50 mM Ambic

Acetone Precipitation:

1. Precipitate protein (30 µg) with four volumes of –20°C acetone vortex, incubate at –80°C for 20 min.
2. Collect the protein by centrifuging at 10,000 rpm for 10 min at 4°C.
3. Discard the supernatant, rinse the pellet with 300 µl of (–20°C) acetone. Centrifuge at 10,000 rpm for 5 minutes at 4°C. Discard the supernatant.
4. Dry the pellet for 5 minutes. Cover samples with foil or a pipette box lid to keep dust out.

Solubilization with ProteaseMAX™ Surfactant/Urea mix:

5. Add 15 µl of 8 M Urea to protein pellet. Bath Sonicate for 5 min.
6. Add 20 µl of 0.2% ProteaseMAX™Surfactant solution. (To make 0.2% solution: 4 µl 1%, 16µl 50 mM Ambic).
7. Mix by shaking on vortexer for 5-10 min.
8. Add 58.5 µl 50 mM Ambic (final volume of 93.5µl).
9. Add 1 µl 0.5 M DTT. Incubate at 50°C for 20 minutes.
10. Add 2.7 µl 0.55 M IAA. Incubate at RT in the dark for 15 min.
11. Add 1µl of 1% ProteaseMAX and 18 µl of 0.1 µg/µl trypsin. Parafilm. Incubate at 37°C for 3 hours.
12. Spin for 10 seconds in microcentrifuge. Add 27 µl 2.5 %TFA (final concentration 0.5%). Mix, and incubate RT 5 min.
13. Dry down in speed vac and clean up with spin columns to remove urea. Use the Pierce PepClean C18 spin columns (Cat#: 89870) for 30 µg of protein.

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**

NOTE: Trypsin 0.1 µg/µl then 18 added so total volume 118

C-18 Spin Column Cleanup

SOLUTIONS**:**

- **Activation Solution** → 50% ACN
- **Equilibration & Wash Solution** → 5% ACN, 0.5% TFA
- **Elution Buffer** → 70% ACN, 0.1% Formic Acid

PROCEDURE:

1. Resuspend sample in 50 µl *Equilibration & Wash Solution*, and bath sonicate for 5-10 minutes.
2. Gently tap bottom of column on bench to settle resin. Remove top and bottom caps. Place column in 2.0 ml eppendorf tube.
3. Add 200 µl *Activation Solution* to rinse walls of column and wet resin.
4. Centrifuge at 1,500 x *g* for 1 minute.
5. Repeat steps 3 & 4. Empty receiver tube.
6. Add 200 µl *Equilibration & Wash Solution*. Centrifuge at 1,500 x *g* for 1 minute. Repeat once. Empty receiver tube.
7. Load resuspended sample on top of resin bed.
8. Place column into a new 1.5 ml receiver tube and centrifuge at 1,500 x *g* for 1 minute.
9. To ensure complete binding, recover flow-through and repeat steps 7 & 8.
10. Place column into 2.0 ml tube used in steps 2-6. Add 200 µl *Equilibration & Wash Solution*. Centrifuge at 1,500 x *g* for 1 minute. Repeat for a total of **4** washes. Be sure to empty the receiver tube after the 2nd wash.
11. Place column in new 1.5 ml receiver tube. Add 20 µl of *Elution Buffer* on top of the resin bed and centrifuge at 1,500 x *g* for 1 minute.
12. Repeat step 11 once more using the same receiver tube.
13. Speed vac to dry down sample
14. Resuspend in LTQ buffer to 1.0 ug/uL for LC-MS/MS.