

in-solution tryptic digest protocol

- Make 0.5M NH_4HCO_3 (40mg/mL)
- Make 15 mg/mL DTT in 0.5M NH_4HCO_3
- Make 1 ug/uL Promega trypsin stock solution by adding 20 uL of resuspension buffer (supplied 5% AcOH) to 20 ug in vial
- Dilute trypsin 50-fold with 0.5M NH_4HCO_3 (20 ng/uL)

FOR USE WITH **0.1mg/mL** PROTEINS (at least 20 uL)

COMBINE:	(for example)
2 parts protein (0.1 mg/mL)	20 uL
1 part 0.5M NH_4HCO_3	10 uL
1 part 15 mg/mL DTT	10 uL
½ part ACN	5 uL
4.5 parts dd H ₂ O	45 uL

HEAT DENATURE 95C, 10 minutes; let cool!

1 part trypsin (20 ng/uL) 10 uL

incubate at 37oC for 4 - 12 hrs.

Quench the reaction with

addition of a 10% volume of 2% TFA, to give ~ 0.2% TFA final.