

1.1 Ettan Digester protocol

1.1.1 Digestion protocol Basic

Suitable for Coomassie and SYPRO Ruby stained gels.

Digestion Solutions:

50 mM Ammonium Bicarbonate / 50% methanol.

20mM Ammonium bicarbonate.

0.1% TFA / 50% Acetonitrile.

Remove liquid: All liquid remaining from spot picking is removed

Wash 1:

- 100 µl volume 50 mM Ammonium Bicarbonate / 50% methanol
- 40 minutes incubation.
- Remove and discard liquid
- Repeat three times

Dry:

Drying samples can be achieved by either adding acetonitrile and letting them air-dry or drying them in a drying module

Digest:

Enzyme:

- 5 µl of 40 ng / µl Trypsin (sequencing grade) prepared in 20 mM Ammonium bicarbonate.
- Incubation time 180 minutes (can be left to overnight) at room temperature or 60 minutes at 37 °C. It is recommended to seal the plates (e.g. Microplate sealing foil, Corning) to prevent evaporation.

Sample transfer:

● *Step 1:*

- 35 µl of 0.1% TFA / 50% Acetonitrile
- Incubate 20 minutes.
- Transfer 35 µl to clean MTP
- Repeat once.

Dry: Samples are completely dried in the drying module (45-60 min).

1.1.2 Digestion protocol Silver

Solutions:

15mM Potassium ferricyanide / 50mM sodium thiosulphate*.
ddH₂O.

50 mM Ammonium bicarbonate / 50% methanol.

0.1% TFA / 50% acetonitrile

20 mM Ammonium bicarbonate.

* This solution should be made by combining equal volumes of 30mM potassium ferricyanide (light sensitive) and 100 mM sodium thiosulphate **immediately prior to digestion** as it is unstable.

Remove liquid: All liquid remaining from spot picking is removed

Wash 1:

- 100 µl 15mM potassium ferricyanide / 50mM sodium thiosulphate
- 20 minutes incubation.
- Remove and discard liquid

Wash 2:

- 100 µl ddH₂O
- 20 minutes incubation.
- Remove and discard liquid
- Repeat four times

Wash 3:

- 100 µl of 50 mM Ammonium Bicarbonate / 50% methanol
- 20 minutes incubation.

Dry: Drying samples can be achieved by either adding acetonitrile and letting them airdry or drying them in a drying module.

Digest:

- *Enzyme:*
 - 5 µl of 40 ng / µl Trypsin prepared in 20 mM Ammonium bicarbonate.
 - Incubation time 180 minutes (can be left to overnight) at room temperature or 60 minutes at 37 °C. It is recommended to seal the plates (e.g. Microplate sealing foil, Corning) to prevent evaporation.

Sample transfer:**Step 1:**

- 35 µl of 0.1% TFA / 50% Acetonitrile
- Incubation time 20 minutes.
- Transfer 35 µl to clean MTP
- Repeat once

Dry: Samples are completely dried in the drying module (45-60 min).