

Digestion of Proteins on PVDF Membranes

This protocol is used in our laboratory on Coomassie or Amido Black stained proteins on PVDF membranes. We routinely use Promega modified trypsin or Endo Lysine C (EKC) from Waco Chemical Co. EKC cleaves after Lysines only and produces larger fragments. Keep in mind that larger fragments may be more hydrophobic and not extract well from the PVDF membrane. We recommend starting with a minimum of 100 pmol of protein for peptides destined to automated Edman sequencing. Less material can be used for mass spec analysis and subsequent protein identification. Although this is still a popular method, it has become superceded by the protocol for digesting proteins in gel slices. For this protocol, as all others, wear gloves and always use the highest quality reagents and chemicals. This is a modification of a protocol shared with us by Steve Tindall, Argo BioAnalytica, Inc, Morris Plains, New Jersey.

DIGESTION BUFFERS:

50 mM Tris pH 9.2, 1% Reduced Triton X-100, 10% Acetonitrile (for EKC)
100 mM ammonium bicarbonate, 1% Reduced Triton X-100, 10% Acetonitrile (for trypsin)

Reducing Solution: Add 10 μ l (1M stock) DTT to 0.99 ml PCS2

Alkylating Solution: Add 1.42 gm acrylamide to 8.2 ml PCS2 (Hewlett Packard)

PCS2: Guanidine-HCL, pH 8.3

REDUCTION & ALKYLATION

Wet membrane with **100%MeOH**

Soak membrane in 100 μ l **Reducing Solution** at **50C** for **10 minutes**

Place membrane in 100 μ l **Alkylating Solution** at **50C** for **5 minutes**

Rinse membrane 1 X with 250 μ l 100 mM Tris pH 9.2

Rinse membrane in 500 μ l **Digestion buffer**

DIGESTION

Cut PVDF membrane into 1 x 1 mm squares (use a fresh razor blade)

Incubate in 20-50 μ l Digestion buffer; mix; 30 min RT (important to use smallest volume possible yet still cover membrane)

Add 0.2 μ gm protease (0.1 μ gm/ μ l); Incubate 40C for 18-24hours

EXTRACTION

Spin sample briefly to collect liquid; sonicate 5 min at 30C

Remove supernatant fluids with gel loading tip to siliconized test tube

Add 50 μ l of digestion buffer, vortex and sonicate 5 min at 30C

Remove supernatant fluids and pool with first supernatant materials

Add 100 μ l of 0.1% TFA to the membranes; vortex and sonicate 5 min at 30C

Remove supernatant fluids and pools with first supernatant materials