

Protocol for Staining of PVDF Membranes

Optimized for N-terminal Edman Sequencing



PROTEIN
ANALYSIS

GUIDELINES

N-terminal Edman sequencing provides the protein amino acid sequence from the N-terminal using the Edman degradation procedure. The method can be used in research for de-novo sequencing of novel proteins, and in biological drug development for identification and confirmation of the N-terminus of a recombinant

protein. Edman sequencing can be performed on samples in solution or from SDS-PAGE separated proteins after electroblotting onto PVDF membranes. Here we describe protocols for electroblotting of proteins from SDS PAGE gels and staining of PVDF blots. The protocols have been optimized to obtain high quality Edman sequencing data.

MATERIALS

Use high quality chemicals and ultra pure water (18.2 Mohm). Wear gloves at all stages and use clean staining trays with lids to avoid keratin contamination of the gels and PVDF. Combine any PVDF membrane and blotting buffer below.

PVDF membranes

Problott (Applied Biosystems)
Immobilon-PSQ (Millipore)
BioTrace (Pall)
Sequi-Blot (BioRad)

Blotting buffers

CAPS buffer: (3-cyclohexylamino-1-propane sulfonic acid) 10 mM CAPS adjusted with NaOH (2N) to pH 11/10% MeOH
Borate buffer: 50 mM sodium borate, pH 9/ 20% MeOH
Tris-Glycine buffer: 25mM Tris, 190 mM Glycine, 0.1 % SDS, pH 8.5/ 20% MeOH
NuPAGE Transfer buffer: Invitrogen

PROCEDURE

1. Run your 1D or 2D gel.
2. Electroblot using one of the PVDF membranes and buffers above.

PONCEAU STAINING

3. If the membrane has dried out, pre-wet it in methanol for a few seconds
4. Stain the membrane in freshly prepared 1% Ponceau S/ 1.0% acetic acid for 2 min.
5. Destain in distilled water until the bands are visible.

COOMASSIE STAINING

3. Stain PVDF with freshly prepared 0.1% coomassie Blue R250 in 40% methanol/1% acetic acid for 30 seconds.
4. Destain with 50% methanol until bands are visible and background clear.
5. Rinse with water.

The PVDF membrane can now be scanned, and protein bands submitted to Pick 'n Post for N-terminal Edman sequencing.

Questions to: info@pick-n-post.com

TROUBLESHOOTING

The PVDF membranes and blotting buffers listed above generally works well for most proteins. However, some proteins may show poor electroblotting efficiency, and the choice of PVDF membrane, blotting buffer and blotting conditions should be optimized. During optimization it is an advantage to stain the gel after blotting and to use 2 layers of PVDF. Some large proteins (above 80 kDa) may be difficult to get out of the gel, and it can help to add 0.1% SDS to the buffer since SDS increases the mobility of the proteins. The same effect can be obtained by omitting MeOH from the buffer, because MeOH strips SDS from the protein.

Some small proteins (below 15 kDa) may move too quickly out of the gel and through to the first PVDF membrane. In that case, SDS should not be used and the MeOH concentration increased to 20%. Also the gel can be pre-soaked in blotting buffer for 5-10 mins before blotting. Choice of blotting buffers with a neutral pH (Tris-Glycine buffers), may be useful for very basic proteins with high isoelectric points. Basic proteins may be positively charged and the PVDF membrane should be placed on the other or on both sides of the gel. Glycine-containing buffers will give high glycine yield in the first Edman cycle, and the PVDF membrane should be washed extensively after staining.