

## Westernblot on PVDF – Membrane

(for N-terminal protein sequencing)

Blot Buffer: 3.09 g Borate /l (50 mM)  
100ml Methanol /l (10% v/v)  
pH 9.0, adjusted with 1M NaOH

Storage: at 4°C

Blot Membrane: Immobilon PSQ Membrane, 0.2µm (Millipore) or  
Problott, 0.2µm (ABI)

Gel 10 min. equilibrate in borate buffer

PVDF Membrane 30 sec. dipping into methanol (important!), following 3 min. in borate buffer.

Blotting Parameters: 25 V, 1.5 h (up to 30kDa) to 2 h (for larger proteins); (for Novex Gels and chambers); Optimization could be necessary.

After blotting stain the membrane 3 min. with Coomassie, followed by destaining of the membrane.

Coomassie: 0.1 % R250  
40% Methanol (v/v)  
10% Acetic acid (v/v)

Filter the solution before usage!

Destaining: 40 % Methanol (v/v)  
10 % Acetic acid (v/v)