proteins from the interleukin-6 dependent plasmacytoma cell line TEPC 2027 (Ward and Simpson, 1991*b*).

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Leonila Fajardo Hermansen, Öyvind Pedersen, and Knut Sletten. Protein Elution and Blotting by Centrifugation. (Department of Biochemistry, University of Oslo, Blindern, N-0316 Oslo 3, Norway)

The elution and blotting of protein from SDS polyacrylamide gels onto a membrane is not an easy task. The most common technique employed is electroblotting, which requires an optimum combination of current and voltage, a buffer with a suitable ionic strength, a temperature control, and a good estimate of transfer time. In an attempt to improve and simplify the blotting procedure, another technique has been designed. It only requires an eluant, a membrane support, a 3.0 kD cut-off dialysis membrane, a blotting receptacle, and a centrifuge (Hettich EBA 3S, table model). The transfer and immobilization of polypeptide from the gel onto the membrane support is achieved by centrifugation, hence it is referred to as *centrifuge-blotting*. The eluant employed is 70% formic acid (Tsugita *et al.*, 1987) and the membrane support is PVDE membrane, which can be directly applied in the microsequencer (Matsudaira, 1987). The centrifuge receptacle assembly is comprised of an outer cylinder which supports the whole structure, and is constructed from a polyoxymethylene material, an inner cylinder which serves as a reservoir for the eluant and is made of tetrafluorethylene, a 12 mm polythene sinter base support which holds the dialysis and PVDF membranes in place, a flat silicon gasket, and a polysterene tube for the collection of the eluate.

The polypeptide separated in the SDS polyacrylamide slab gel (10–15% T) (Laemmli, 1970) and visualized with 1.0 M KCl (Nelles and Bamburg, 1976; Bergman and Jörnvall, 1987) has been excised and positioned on top of the PVDF membrane in the centrifuge receptable. About 1.0 ml eluant is added and the gel bit is then allowed to equilibrate and swell for around 10 min. Centrifugation of the receptacle is then performed at about 1000 *g* for 1 hr, and the speed is maintained at this level to facilitate interaction between the protein and the PVDF membrane. By spinning the receptacle, the eluant diffuses through the gel matrix and carries the polypeptides which then adhere on the PVDF membrane. It is assumed that the swelling of the gel in 70% formic acid and the centrifugation process hasten the diffusion of the protein from the polyacrylamide gel. Unbound proteins can be recovered from the 3.0 kD cut-off dialysis membrane which acts as a sieve. Different proteins with molecular mass from 13–67 kD have been centrifuge-blotted. So far, the overall yield (after weighing, electrophoresis, blotting, amino acid hydrolysis, and extraction of the protein from the membrane) obtained is about 10–20%. This recovery varies, depending on the protein type and size. Direct microsequencing of a 13 or 14 kD protein immobilized onto the PVDF membrane gave an initial yield of about 10–15%, and 14–19 degradation cycles have been attained with a repetitive yield of about 92%.

There are several factors which must be considered to optimize the centrifuge-blotting technique, such as: speed and time of centrifugation; type and amount of eluant; effect of piling up gel bits in several layers; amount and type of protein applied; and type of membrane.

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The introduction of polyvinylidene difluoride membrane (PVDF) for blotting of proteins separated by SDS poly-