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Purification of a glycoprotein (tissue plasminogen activator (t-PA)) by means of affinity interaction using novel designed membrane adsorber spin columns

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1 Introduction

The recently introduced antibody coupling kit, developed by Vivascience AG, is optimised for antibody immobilisation in a spin column format. The kit contains spin columns with epoxy-activated membranes and coupling buffers. All coupling steps and the following purification are performed in a centrifuge. The spin columns can be used to couple small amounts of antibody (approx. 0,4 - 0,6 mg). t-PA and the antibody anti-t-PA were used as a model system for antibody coupling and a following antigen purification. First anti-t-PA was immobilized on epoxy-activated membranes and t-PA was isolated from cell culture medium.
The results were verified by SDS-PAGE gels.

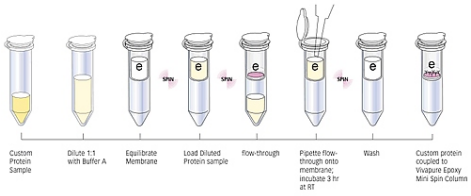
2 t-PA

Tissue plasminogen activator (t-PA) is a 70 kDa glycoprotein with 10% of carbohydrate.
t-PA binds with high affinity to fibrin clots together with the protein plasminogen.
In complex, t-PA catalyses the transfer of plasminogen into plasmin, which inhibits the coagulation of blood and dissolves clots. t-PA is therapeutically used for the treatment of heart stroke patients.

3 Materials and Methods 1

Antibody coupling

·pre-wet epoxy activated spin column with 400 µl 0,5 M KPP buffer pH 8,0; spin at 200 g for 5 min
·dilute anti-t-PA in PBS (2 mg/ml) 1:1 with 1 M KPP buffer pH 8,0; load 400 µl coupling solution onto the prepared spin column; spin 5-10 min at 500 g
·pipette flow through onto the membrane again, leave spin column to incubate for 3 h at room temperature and spin again
·wash 2 x with 0,5 M KPP buffer, 2x with 1 M NaCl in 0,5 M KPP buffer and 2 x with 0,5 M KPP buffer again; pH 8,0; spin each time at 2000 g for 5 min



4 Materials and Methods 2

Antigen purification

·pipette 400 µl 0,5 M NaCl in 10 mM KPP buffer pH 7,4 onto the affinity membrane and centrifuge at 800 g for 5 min
·load the spin column with 400 µl antigen solution (0,2 mg/ml) and spin 7 min at 400 g
·reload the flow through onto the membrane and repeat centrifugation
·wash with 400 µl 0,5 M NaCl in 10 mM KPP pH 7,4 and spin at 800 g for 5 min
·elute 2x with 400 µl 0,1 M glycine/HCl pH 2,8 and spin at 800 g for 5 min
·to maintain antigen activity, the elution fraction is neutralised with 50 µl 1 M Tris Buffer pH 9

5 SDS-PAGE

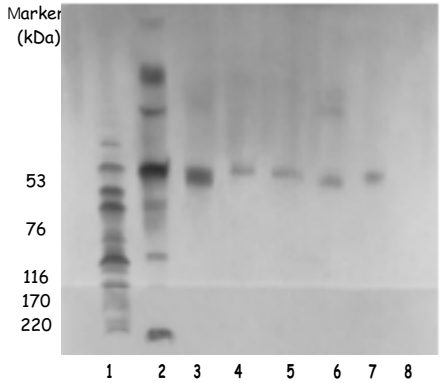


Fig. 1: 12% SDS-gel

1. marker
2. anti-t-PA stock solution
3. t-PA stock solution
4. flow through anti-t-PA
5. 1st wash-step anti-t-PA
6. flow through t-PA
7. 1st elution
8. 2nd elution

6 Results and Discussion

Anti-t-PA was immobilized on the epoxy-activated membranes. In a second step the antigen t-PA was successfully purified from cell culture medium.
This result demonstrates, that there was no significant loss of biological activity of the antibody due to the immobilisation step.

VIVASCIENCE