ACTIVATION, DERIVATIZATION OF AND PROTEIN IMMOBILIZATION ON HC OR OTHER CARBOXYL FUNCTIONALIZED SLIDES

Instructions for use

GENERAL INFORMATION

HC slides are coated with a dense polycarboxylate hydrogel which has a brush-like linear structure. Upon contact with water, this layer swells to 1–2 µm thickness and can electrostatically immobilize up to 80 ng protein/mm^2. The accumulation of such relatively high protein densities within the hydrogel is achieved by electrostatic attraction between the negatively charged hydrogel and the – at sufficiently low pH – positively charged proteins. These forces occur at low ionic strengths and at a low pH only, so the choice of the correct immobilization buffer is crucial. If the polycarboxylate hydrogel has been activated with NHS prior, the preconcentrated proteins will be covalently bound. Non-specific binding is low under physiological conditions, so it is not necessary to block the surface after immobilization.

MATERIALS

- Carboxyl functionalized slide.
- Protein solution: 0.1–5 mg/mL in coupling buffer. Sensitive proteins might require addition of small amounts (less then 0.1%) protective agents such as saccharides or low molecular weight polyethylene glycol (PEG) to preserve their activity.
- Coupling buffer: Optimal is 2 mM sodium acetate pH 4.0–5.5 in order to ensure a positive net charge of the protein which is required for electrostatic interaction with the negatively charged surface. Other buffer systems such as MES, phosphate, borate and carbonate buffers as well as ionic strengths (NaCl) from 5 mM to 0.1 M sometimes work as well, but give significantly lower immobilization yields
- Elution buffer: 1 M sodium chloride + 0,1 M sodium carbonate buffer pH 10
- Washing buffer: 5 mM acetic acid
- Quenching buffer: 0.5 M ethanolamine hydrochloride pH 8.5
- Activation mix: 0.5 % (w/v) solid N-ethyl-N'-dimethylaminopropyl)-carbodiimide) (EDC) in 0.1 M N-hydroxysuccinimide (NHS), 0.05 M 2-(N-morpholino) ethane sulfonic acid (MES) buffer pH 5.5.*

*) EDC solutions hydrolyze within less than one hour and are not active anymore. We recommend freezing aliquots of the NHS / MES buffer. Immediately before use dissolve the required quantity solid EDC in the MES/NHS buffer.

ACTIVATION PROTOCOL

1. Place carboxyl slides in a clean, suitably sized receptacle and shake 5 min with elution buffer.
2. Rinse 2–3 times with dd water. Prepare the activation mix.
3. For a sufficient degree of activation, incubate the surfaces 15 min with the activation mix. If a lower level of immobilization is required, choose a shorter activation time (1, 2 or 5 min). Once activated, the slides should be rinsed and spotted as fast as possible.
4. Rinse 3 times with washing buffer and dry the slides with a sharp jet of clean compressed air or nitrogen or a quick spin. It is essential that no droplets dry on the surface as even dd water leaves contaminations behind which might interfere with later processing steps.

BULK DERIVATISATION

After resp. during activation, the carboxyl groups can be derivatised further with amine containing ligands. If the ligands are small and bear no carboxyl functionalities, they can be added to the activation mix directly up to a concentration of 0.2 M. Otherwise the activated slides must be first washed with washing buffer and can then be incubated in an at least 5 mM solution of the corresponding ligand in 0.1 M MES pH 6.0. If the ligand is a protein, use appropriate incubation buffer for electrostatic preconcentration as outlined above. A typical reaction time would be 2–5 hrs. Continue with step 3 of the spotting protocol.

SPOTTING PROTOCOL

1. Place the slides in a spotter and spot the protein solutions. Recommended humidity: 100 %. A lower humidity can result in smaller spots, an up to 50 % lower immobilization level and denaturation of the spotted proteins. The spotting area is 60x 20 mm, so at least 2.5 mm to the right and left and 7.5 mm to the upper and lower end should be left blank. The typical standard deviation over the slide surface is 5–7 %.
2. Incubate at 100% humidity over 2–5 hrs.
3. React remaining active NHS esters 30–120 min with quenching buffer.
4. Optional: Block with suitable blocking buffer.
5. Rinse with dd. water and dry the slides with a quick spin or a sharp jet of clean compressed air/ nitrogen. Sensitive proteins might lose their activity upon drying without protective additives. Store the dried slides at 4 °C under nitrogen (50–80 % humidity) in the dark.

LIGAND INCUBATION

Buffer composition and incubation time depend on the nature of the immobilizate and the corresponding analytes. Generally, physiological buffers, such as protein containing PBS or Tris and an incubation time of at least 2 h gave good results. To minimize potential ionic interactions between the analyte matrix and charged hydrogel, we recommend to wash after the incubation 2x 10 min with PBS + 0.2 % Tween and another 2x 10 min with phosphate buffered 1 M NaCl pH 8.0. Depending on the affinity of the ligand/analyte pair, alternative conditions may apply.

During ligand incubation, but also during quenching / blocking, ensure that convection is sufficient, as an efficient transport of the analyte resp. ligand into and from the hydrogel enhances the overall signal, leads to better defined spot shapes and optimal homogeneity over the slide surface.

Finally, rinse again with dd. water and dry the slides with a quick spin or a sharp jet of clean compressed air/nitrogen.

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