PROTEIN IMMOBILIZATION ON NHS ACTIVATED HYDROGEL (HCX) SLIDES

Instructions for use

GENERAL INFORMATION

Preactivated HCX slides are ready to spot and coated with a dense carboxyl functionalized hydrogel which is partially NHS activated. Upon contact with water and hydrolysis of the NHS esters, this layer swells to 1–2 µm thickness and can immobilize up to 80 ng protein / mm². Nonspecific binding is low under physiological conditions, so blocking the surface after spotting is usually not required. The capability of the hydrogel surface to rapidly adsorb liquids results in a defined shape of the spotted droplets despite the surface has a low contact angle. The quenched (hydrolyzed) hydrogel surface is very hydrophilic and has a contact angle of around zero.

Note: For good results it is essential to use the correct spotting buffer as indicated below.

MATERIALS

- HCX Slide.
- Protein solution: 0.1–5 mg/mL in spotting buffer. As the hydrogel surface can immobilize relatively high protein amounts, we recommend to work with protein concentrations of at least 0.5 mg/mL. 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. 0.1–0.5 % Alginate can be used to modify the viscosity of the spotting solutions, if necessary.
- Spotting buffer: 5 mM acetate buffer pH 5.0. MES, phosphate, borate and carbonate buffers pH 5–9 and ionic strengths (NaCl) from 5–100 mM can be used as well but give significantly lower coupling yields. Do not use amine containing buffers, such as Tris and avoid addition of azide, as these nucleophiles crossreact with the active NHS esters on the slide surface.
- Quenching buffer: 1 M ethanolamine hydrochloride pH 8.5.

SPOTTING PROTOCOL

Note: The hydrogel layer on the slide surface repels not only proteins (low background) but also paint and glue. Therefore, marker pens and labels shouldn't be used as they are easily washed off.

1. If not already done, adjust the spotter to a slide thickness of 1.00 mm.
2. For rehydration of the hydrogel immerse the slides in dd water and incubate for 15 min. Then dry the slides with a quick spin or a sharp jet of clean compressed air/nitrogen. It is essential that no droplets dry on the surface as even dd water leaves contaminations behind which might interfere with later processing steps.
3. Place the slides in the spotter and spot the protein solutions. Recommended humidity: 80–100 %. A lower humidity can result in merged spots, a lower immobilisation level and denaturation of the spotted proteins. The spotting area is 60 x 21 mm, so at least 2.0 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 %.
4. Let the spots dry and incubate at 80–100 % humidity over 2–5 hrs.
5. React remaining active NHS esters 30–60 min with quenching buffer.
6. Optional but not recommended: Block with suitable blocking buffer.
7. Rinse with dd. water and dry the slides with a quick spin or a sharp jet of clean compressed air/nitrogen. It is essential that no droplets dry on the surface as even dd water leaves contaminations behind which might interfere with later processing steps. Sensitive proteins might lose their activity upon drying without protective additives. To avoid such deactivation, XanTec's Stabilization Buffer can be used. Store the dried slides at 4 °C under nitrogen 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 maximize the specificity of the assay, 2x 10 min washes with PBS + 0.2 % Tween can be useful. Electrostatically adsorbed proteins can be removed with high ionic strength buffers such as phosphate buffered 1 M NaCl pH 8.0 if the ligand-analyte interaction tolerates such a condition. 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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