0.1 Bead functionalization

0.1.1 Covalent coupling of EGF to carboxylate modified beads

Reagents

- Latex beads, 0.885 μm, Carboxylate-Modified Polysterene (Sigma) 2mL $79.6 # CLB-9
- EDAC (Pierce) 5g # 22980 MW 191.7
- NHS (Pierce)✓ FW 115.09
- BME (Sigma)
- MES (Sigma) ✓ FW 195.2
- Hydroxylamine (Sigma) w/ hcl (HCl, FW = 69.49)
- EGF, Murine Submaxillary Glands (Calbiochem) 100µg $135 # 324851

Protocol (modified from Pierce)

- Make 1 ml 1% bead suspension in 50 mM MES at pH 6.1
- Prepare stock solutions of EDC (200 mM) and NHS (500 mM)
- **Esterification:** Add 10 μl of the EDC and NHS stock solutions to the beads ($\Rightarrow$ 2 mM EDC and 4 mM NHS). Incubate 15 minutes while rotating gently.
- In the meantime prepare 1 ml sodium bicarbonate buffer at pH 8.3. Add EGF to a final concentration of 1 μg/ml.
- **Quench EDC:** Add 1,4 μl BME to the beads after the incubation.
- Wash the beads. (Quick, because the NHS hydrolyses with $t_{1/2}$=30 min.)
- **Couple the EGF:** After the last wash, take up the beads in the EGF solution at pH 8.3
- Incubate 30 minutes at room temperature while rotating gently.
- **Quench reaction:** Add 10 μl of 1 M Hydroxylamine. ($\Rightarrow$ Any unreacted NHS is hydrolysed.)
- Wash with PBS a lot.
- The beads can be stored at -20° in a 50% glycerol solution.

Bastiaens
Philippe
Science.
EGFR labeling

Jovin protocol: EGF-coated magnetic beads.

Sera-Mag carboxylate-modified microparticles.
composition: styrene/acrylic acid polymer + Fe_3O_4 (magnetite) coat + surface CO_2^- groups
size: 0.768 μM
concentration: 5% solids (50 mg/mL) in H_2O + 0.05% NaN_3
storage: 4°. do not freeze
Seradyn.com, cat # 2947660502.50

prepare solutions:
500mM MES pH ~~6.0~~ 5.0. Store 4°C. (FW 195.2 ⇒ 9.76g/100mL, pH w/ 1N NaOH)

500 ~~100~~ mM NHS. Make fresh (FW 115 ⇒ 575mg/10mL) ... 4°C

500mM EDC. Make fresh (FW 192 ⇒ 960mg/10mL) -20°C, 4°C halfway

500mM Na_2HPO_4 pH 8 (FW 142 ⇒ 7.1g/100mL, pH w/ 1N HCl)

1M ethanolamine: (FW 61.08 ⇒ 611mg/10mL)

Preparation of liganded microspheres

Natural murine EGF was purchased from IC Chemikalien (Ismaning, Germany). Carboxy-functionalized super-paramagnetic 1 μm beads (SERA-MAG, Seradyn, Indianapolis, USA) were activated with 0.1 M sulfo-NHS (N-hydroxysulfosuccinimide; Pierce, Rockford, USA), 0.1 M EDC (1, ethyl(-3-[3-dimethylaminopropyl]carbodiimide hydrochloride; Pierce) in 0.1 M MES (Sigma, Deisenhofen, Germany) buffer, pH 5, for 1 hour at room temperature. After washing twice in 0.1 M MES, the microspheres were equilibrated in coupling buffer (0.1 M sodium phosphate, pH 8). The coupling reaction was carried out at 4°C overnight with 50 μg EGF in 30 μl coupling buffer per 6 μl of the 5% bead slurry with constant agitation. Beads used as negative controls in immunofluorescence experiments were incubated with ethanolamine or BSA (Sigma). Finally, the beads were washed twice with coupling buffer and then thoroughly with PBS after quenching remaining activated groups with 1 M ethanolamine for 2 hours at room temperature. The microspheres were stored in PBS with 0.1% sodium azide. The presence of intact EGF on the surface of the microspheres was demonstrated in a bead agglutination assay (Dezelic et al., 1971) with an anti-murine EGF rabbit polyclonal antibody (Sigma). A nonspecific antibody was inactive

T.M. Jovin
J. Cell Science 114, 2437 (2001)

Test beads using fixation protocol

1. K+ cells, untransfected, starved 24 hr
2. In HBSS (1.5 mL) ± EGF (5 uL) or ± EGF beads (Jovin, 25 uL)
3. 10 min @ RT for sEGF, 20 min for EGF beads
4. Cool to 4°C
5. Rinse 2x w/ 4°C PBS
6. Rinse 1x w/ -20°C MeOH (100%)
7. -20° MeOH, 30 min
8. Wash 2X w/ PBS, 4°
9. Add 1mL 4° blocking buffer (1 mg/mL BSA + 0.05% Triton in PBS)
   Incubate for 30 min @ 4°. Remove liquid
10. Add 5 ug/mL 4G10 α-pY mouse in blocking buffer
    Incubate 4° 1 hr
11. Wash (on rotisserie 5 min) w/ PBS @ 4°C 4x
12. Add FITC-conj α-mouse goat
    Incubate 1 hr 4°
13. Wash 4x w/ 4° PBS
14. Store in PBS @ 4°C. up to 24 hr. (pg 14.6.2 of red book)

NB: Jovin uses PY99 α-pY Ab from Santa Cruz.