

Day 3 : Elution of antibodies

Perform elution of antibodies in a coldroom (4°C). Wash 3x with PBS, then finally resuspend beads in 10 ml of PBS.

Load beads into a disposable plastic column (for example BioRad Poly-Prep chromatography columns). Wash with 10x volumes of PBS (~1 h) until OD 280 = 0. Use PBS as the blank in the spectrophotometer.

Elute the bound antibodies with 200 mM glycine pH 2.8. Collect 1 ml aliquots into tubes containing 27 µl of 3 M TRIS-HCl, pH 8.8 and 100 µl of 3 M KCl. Measure OD 280 of fractions.

Optional : dialyse antibodies overnight at 4°C against PBS. Use dialysis tubing with a cut off of 12000-14000 mw. Exchange buffer three times.

Keep a 20 µl aliquot of the antibodies at 4°C for testing. Snap freeze aliquots of antibodies in liquid nitrogen, then store at -20°C.

Wash beads on column with 200 mM glycine pH 2.2 until OD = 0. Wash beads 3x with PBS, then store at 4°C in PBS / 0.01% NaN₃.

Buffers

Carbonate buffer

Stock 0.5 M Na₂CO₃ 53 g/l pH ~ 11
Stock 0.5 M NaHCO₃ 42 g/l pH ~ 8

Start with Na₂CO₃ pH ~ 11 (minimum volume ~ 7 ml). Add NaHCO₃ pH ~ 8 (to approximately 200 ml) until pH is 8.3. This solution is 0.5M carbonate buffer.

Coupling buffer

0.1M carbonate buffer
0.5M NaCl
pH 8.3

Stock 0.5 M carbonate buffer pH 8.3 (above)	100	ml (0.1 M final)
Stock 3 M NaCl	83.3	ml (0.5 M final)

Total volume	500	ml

Acetate buffer

0.1 M Na acetate
0.5 M NaCl
pH 4.0 (use acetic acid)

TRIS buffer

0.1 M TRIS-HCl
0.5 M NaCl
pH 8.0 (use HCl)