Fibronectin Buffers

Regeneration Buffers

High pH (500 ml pH 8.5)
- 450 ml MilliQ Water
- 6.05 g Trisma Base (0.1M = 12.1 g/L)
- 14.61 g NaCl (0.5M = 29.22 g/L)
- Dissolve, pH to 8.5, bring final volume up to 500 ml

Low pH (500 ml pH 4.5)
- 450 ml MilliQ Water
- 4.1 g Sodium Acetate (0.1M = 8.2 g/L)
- 14.61 g NaCl (0.5M)
- Dissolve, pH to 4.5, bring final volume up to 500 ml

Wash Buffers

Wash Buffer (1) - 2, 1L bottles
- 880 ml MilliQ Water per bottle
- 100 ml 10x PBS per bottle (1x PBS final)
- 10 ml* 0.2 M EDTA, pH 8.0 per bottle (2 mM EDTA final) (see below for details)
- 10 ml*^ 0.1 M PMSF in 100% Ethanol **ADD JUST BEFORE USE!** (1 mM final) (See below for details)
- Final Volume - 1 L per bottle
- *Add EDTA the same day as being used. First 1 L bottle will be used day 1 and second 1L bottle will be used on day 2. Add Accordingly. 200 ml of Wash Buffer (1) will be removed and used in Wash Buffer (2). When adding EDTA and PMSF, change added volume to 8 ml each into the 1L bottle that buffer was removed from, adding the additional 2 ml to Wash Buffer (2) instead.
- ^Add PMSF immediately before use. Remember that one bottle of Wash Buffer (1) will have 200 ml less volume than the other. Only add 8 ml of PMSF to that bottle.

Wash Buffer (2)
- 196 ml Wash Buffer (1)
- 11.68 g NaCl (1 M)
- 2 ml 0.2 M EDTA, pH 8.0 (2 mM EDTA final) (from 10 ml volume to be added to Buffer 1)
- 2 ml 0.1 M PMSF in 100% ethanol (1 mM PMSF final) (same as EDTA)
- Final volume - 200 ml

Dialysis/Elution Buffer

4x Dialysis Buffer - 1 L to be added to 3 L MilliQ Water
- 800 ml MilliQ Water
- 8.84 g Caps (40 mM) - final 4 L concentration 10 mM
- 35.06 g NaCl (0.6 M NaCl) - final 4 L concentration 0.15 M
- 40 ml 0.2 M EDTA, pH 8.0 (8 mM EDTA) - final 4 L concentration 2 mM
- Bring volume to 900 ml with MilliQ Water
- pH to 11.0 using NaOH
- Final volume to 980 ml
- Right before use, add 20 ml of 0.4 M PMSF (see below)

Elution Buffer - 100 ml from 4x Dialysis buffer
25 ml MilliQ Water
With stir bar and hot plate, warm water
24.02 g Urea (4 M = 240.24 g/L) - add just as water is warming.
Dissolve with low heat. Do not boil. Volume will be around 50 ml
Add 25 ml of 4x dialysis buffer (no PMSF)
Bring volume to around 75 ml
pH to 11.0 using NaOH - should be very close
Bring volume to 100 ml
Add 0.5 ml PMSF right before use

PMSF/EDTA Stock Solutions