Pepsin Actigel

Pepsin Actigel was developed for the production of $F(ab')_2$ fragments from IgG molecules. It is intended to be used with Sterogene Bioseparations' Protein A media. Pepsin has an optimum pH range between 6 and 7 and requires 20 mM Cysteine for activation.

Buffers:

Digestion Buffer:	20 mM Acetate, pH 4.5
Pepsin Actigel Storage Buffer:	0.1M Acetate, 50% Glycerol, 0.05% Azide, pH 4.5
Pepsin Actigel Equilibration Buffer:	20mM Tris, pH 8.5
Pepsin Elution Buffer:	0.1M Glycine, pH2.8 or 0.1M Citrate, pH 2.7
Neutralization Buffer:	1M Tris base
Pepsin Storage Buffer:	20% Ethanol

Protocol:

1. For every 20 mg of IgG use 1 mL Pepsin Actigel in 3 mL Digestion Buffer (optimal IgG concentration is 5 mg/mL in Digestion Buffer + resin).
2. Optimize digestion time, between 2-18 hours; 6 hours is typical for IgG at 5 mg/mL at 37 °C.
3. Remove supernatant and adjust pH to 8.5 with Tris base.
4. Regenerate Pepsin Actigel with 10 volumes Digestion and Regeneration Buffer.
5. Load on Protein A medium and equilibrated with 20 mM Tris, pH 8.5.
6. Collect fractions and assay for $F(ab')_2$ – 3 bed volumes should be sufficient.
7. Store $F(ab')_2$ fragments under conditions optimized for the specific fragments.
8. Elute bound material from Protein A medium until the absorbance reaches baseline.
9. Re-equilibrate Protein A medium in Equilibration Buffer until pH of eluent is 8-8.5.
10. Store Pepsin Actigel and Protein A medium in the appropriate storage buffer above.