Papain Actigel

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

Buffers:

Digestion Buffer:	20 mM Phosphate, 20 mM Cysteine, 10 mM EDTA, pH 7
Enzyme Regeneration Buffer:	0.1M Phosphate, 2 mM EDTA, 10 mM Dithiothreitol, pH 6.8
Papain Actigel Storage Buffer:	0.1M Acetate, 50% Glycerol, pH 4.5
Papain Equilibration Buffer:	20mM Tris, pH 8.5
Papain Elution Buffer:	0.1M Glycine, pH 2.8 or 0.1M Citrate, pH 2.7
Neutralization Buffer:	1M Tris base
Papain Storage Buffer:	20% Ethanol

Protocol: Regenerate Papain Actigel before first use and after > 1 month of storage.

1. Wash Papain Actigel with 10 bed volumes of deionized (DI) water.
2. Add 1.5 volumes of Regeneration Buffer and mix for one hour at room temperature.
3. Wash with 10 bed volumes of DI water.
4. Store in Papain Actigel Storage Buffer or equilibrate in 1.5 volumes Digestion Buffer.

Digestion

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