

Immobilized Pepsin Protocol and Product Information Sheet

Product Category: Immobilization Resins
Catalog Number(s): g4106-2ml, [g4106-5ml](#), g4106-15ml
Product Name: Immobilized Pepsin

Immobilized Pepsin

Immobilized Pepsin 2 ml (g4106-2ml), 5 ml (g4106-5ml), or 15 ml (g4106-15ml) of settled gel is supplied as a 50% slurry in pH 4.5 buffer containing 50% glycerol, 100 mM Sodium Acetate, and 0.05% NaN₃.

Gel Support: Crosslinked 6% beaded agarose.

Storage: Upon receipt store at 4°C (shipped at ambient temperature).

Protocol for Antibody Digestion and F(ab')₂ Fragmentation with Immobilized Pepsin

Note: Optimization of Immobilized Pepsin protocol is required for specific applications, antibody species, and subclasses. The reaction rate will be increased by increasing the enzyme to protein substrate ratio and incubation temperature. Recommended reaction conditions are pH 1-3, 20°C to 37°C.

1. Make a digestion buffer consisting of 0.2 M Sodium Acetate, pH 4.4 (or other suitable buffer).
2. Suspend the Immobilized Pepsin with gentle agitation and aliquot 250 ul of the 50% slurry into a 15 ml tube using a wide bore pipette tip.
3. Wash Immobilized Pepsin by adding 4 ml digestion buffer and swirl gently to mix. Allow the resin to separate from the buffer by centrifugation (~800-1,000 x g for 2-4 minutes) or by using a serum separator and discard the buffer. Repeat wash step then resuspend Immobilized Pepsin in 500 ul of the digestion buffer.
4. If IgG is salt free, then dissolve up to 10 mg IgG/ml digestion buffer, otherwise dialyze IgG with digestion buffer and concentrate to 10 mg/ml.
5. Add 1 ml prepared IgG to washed resin from step 3. Incubate the reaction mixture in a rapidly shaking water bath for 4-5 hours at 37°C.
6. Separate the Immobilized Pepsin gel from the digestion mixture as noted in step 3. Retain supernatant in a new tube as your pepsin-digested protein sample.
7. Maximum fragment recovery can be obtained by washing Immobilized Pepsin with 1-2 ml of 0.01 M Tris-HCl, pH 7.5. Add wash to digest recovered in step 6.