



## Immobilized Trypsin Protocol and Product Information Sheet

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Product Category: Immobilization Resins  
Catalog Number(s): [g4107-2ml](#), g4107-5ml, g4107-15ml  
Product Name: Immobilized Trypsin

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### Immobilized Trypsin

Immobilized Trypsin 2 ml (g4107-2ml), 5 ml (g4107-5ml), or 15 ml (g4107-15ml) of settled gel is supplied as a 50% slurry in buffer containing 0.05% NaN<sub>3</sub> and 50% glycerol.

Gel Support: Crosslinked 6% beaded agarose.

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

### Immobilized Trypsin Digestion Protocol

**Note:** Optimization of Immobilized Trypsin protocol is required for specific applications. Recommended reaction conditions are pH 7.5 to 9.0 at 37°C. The reaction rate will be increased by increasing the enzyme to protein substrate ratio and incubation temperature. For example, a typical digestion of 4 hours to overnight can be achieved using a ratio of 1:25 enzyme to protein substrate, while it is recommended to use 1:10 enzyme to protein substrate for accelerated trypsin digestion (0.5 to 1 hour).

1. Make 100 mM Ammonium Bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>), pH 8 (or other suitable buffer such as 100 mM Triethylamine Acetate, pH 8) for use as a digestion buffer.
2. Dissolve 1 mg of your protein sample in 500 µl digestion buffer.
3. Wash 0.15 to 0.3 ml of the Immobilized Trypsin with 3 x 0.5 ml of digestion buffer. Separate the gel from the wash buffer after each wash by centrifugation or by using a serum separator. Discard buffer after each wash.
4. Re-suspend the washed Immobilized Trypsin gel in ~200 µl of digestion buffer, and add the Immobilized Trypsin to your protein sample.
5. Incubate enzyme/substrate mixture in a shaking water bath for 2-18 hours at 37°C with rapid agitation.
6. Separate the Immobilized Trypsin resin from the digestion mixture as noted in step 3. Retain supernatant as your trypsin-digested protein sample.