



## Immobilized Chymotrypsin Protocol and Product Information Sheet

---

Product Category: Immobilization Resins  
Catalog Number(s): [g4105-2ml](#), g4105-5ml, g4105-15ml  
Product Name: Immobilized Chymotrypsin

---

### Immobilized Chymotrypsin

Immobilized Chymotrypsin 2 ml (g4105-2ml), 5 ml (g4105-5ml), or 15 ml (g4105-15ml) of settled gel is supplied as a 50% slurry in buffer containing 0.02% 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 Chymotrypsin Digestion Protocol

**Note:** Optimization of Immobilized Chymotrypsin 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 chymotrypsin digestion (0.5 to 1 hour).

1. Make a digestion buffer consisting of 0.08 M Tris-HCl, 0.1 M Calcium Chloride, pH 7.8 (or other suitable buffer such as 0.1 M Ammonium Bicarbonate, 0.01 M Calcium Chloride, pH 8.0).
2. Dissolve 1 mg of your protein sample in 200 µl digestion buffer.
3. Wash 0.2 to 0.3 ml of the Immobilized Chymotrypsin with 3 x 0.5 ml of digestion buffer. Separate the gel from the buffer after each wash by centrifugation (~2,000 RPM) or by using a serum separator. Discard buffer after each wash.
4. Re-suspend the washed Immobilized Chymotrypsin gel in ~200 µl of digestion buffer, and add the Immobilized Chymotrypsin 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 Chymotrypsin gel from the digestion mixture as noted in step 3. Retain supernatant as your chymotrypsin-digested protein sample.