

# Immobilized Papain

20341

0107.3

Number	Description
20341	<p><b>Immobilized Papain</b>, 5mL of settled gel</p> <p>Support: Cross-linked 6% beaded agarose</p> <p>Supplied: 50% slurry (10mL total volume) in 50% glycerol, 0.1M sodium acetate, pH 4.4 containing 0.05% sodium azide</p> <p>Activity: 16-40 BAEE units per milligram of papain before immobilization</p> <p>Loading: 250µg papain/mL of settled gel</p> <p><b>Storage:</b> Upon receipt store at 4°C. Product is shipped at ambient temperature.</p>

## Introduction

Thermo Scientific Immobilized Papain is convenient for producing Fab and Fc fragments from a variety of IgG species. Papain is a nonspecific, thiol-endopeptidase that has a sulfhydryl group in the active site, which must be in the reduced form for activity. When IgG molecules are incubated with papain in the presence of cysteine, one or more peptide bonds in the hinge region are split, producing three fragments of similar size: two Fab fragments and one Fc fragment. The Fc fragments can be separated from the Fab fragments using immobilized Protein A or by ion exchange chromatography.

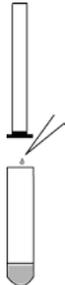
The 50,000Da Fc fragment is often used for determining specificity of the Fc receptors without interference from antigen binding.<sup>1</sup> Interactions of immunoglobulin molecules with Fc receptors help regulate the physiology of the cell and alteration in Fc receptor function may cause onset or progression of certain diseases. By following the interaction of Fc receptors and immunoglobulins, the phenomenon of cell surface recognition mediation and activation can be studied.<sup>2</sup>

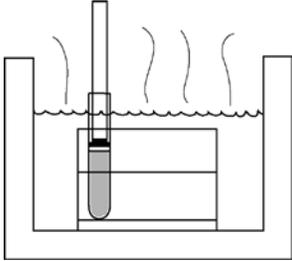
Fab fragments are useful in immunohistochemical studies because the fragments penetrate the tissue better than intact IgG. Also, nonspecific binding from Fc receptors is avoided by using only the Fab portion of the immunoglobulin.

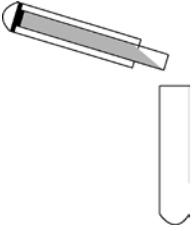
Using Immobilized Papain allows generation of fragments without contamination of the final preparation with enzyme. Immobilization increases enzyme stability against heat denaturation and autolysis and results in longer maintenance of activity. Cleavage can be regulated via digestion time or flow rate through a column. Immobilized Papain is effective with small volumes of dilute proteins and yields reproducible digests.

## Procedure Summary

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1. Prepare the IgG sample and reagents.
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2. Equilibrate the Immobilized Papain.
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3. Add the prepared IgG sample and incubate at 37°C.
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4. Separate the crude digest from the Immobilized Papain.
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5. Separate the Fc fragments from the Fab fragments.

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## Procedure for IgG Digestion to Generate Fab Fragments

This protocol is for digestion of polyclonal mouse and human IgG. Optimization will be required when attempting digestion of IgG from species other than mouse or human. Digestion of various IgG species with non-immobilized and Immobilized Papain is reported in the literature (see Table 1 in the Additional Information Section). Some species of IgG, such as rabbit, are difficult to digest and require a higher ratio of enzyme to substrate (Enzyme: Substrate, 1:10 w/w)<sup>3</sup> than suggested in the following protocol (Enzyme:Substrate, 1:160 w/w), while other species of IgG, such as rat, are easily denatured by this enzyme requiring less enzyme or less time for digestion.

### A. Additional Materials Required

- Test tubes or other reaction vessels
  - Resin separators (Product No. 69710) or a centrifuge to separate the crude digest from the Immobilized Papain
  - Sample Buffer: 20mM sodium phosphate, 10mM EDTA; pH 7.0
  - Cysteine•HCl (Product No. 44889)
  - Shaking water bath capable of maintaining 37°C
  - Immobilized Protein A (Product No. 20356) or an ion exchange column to purify Fab or Fc fragments
- Note:** Do not use immobilized Protein G because Fabs, as well as Fc fragments, have some affinity for Protein G.
- 10mM Tris•HCl, pH 7.5

### B. Preparation

1. If the IgG is purified and lyophilized, proceed to Step 2. If the IgG is in solution, dialyze against Sample Buffer. Concentrate IgG to approximately 20mg/mL. Lower concentrations will require a higher enzyme to substrate ratio or a longer digestion time.

**Important:** Proper sample preparation is essential for successful fragment generation.

2. Just before use, prepare Digestion Buffer by adding cysteine•HCl to a final concentration of 20mM to the Sample Buffer and adjusting the pH to 7.0.
3. Mix the Immobilized Papain by inversion or gentle shaking to obtain an even suspension. Add 0.5mL of the 50% Immobilized Papain slurry to a glass test tube or other suitable reaction vessel. To ensure proper gel slurry dispensing, use a wide bore or cut pipette tip.
4. To equilibrate the gel, add 4.0mL of Digestion Buffer to the gel slurry. Separate the gel from the buffer with a resin separator or by centrifugation. Repeat this wash procedure with another 4.0mL of buffer. Discard both washes.
5. Resuspend the gel in 0.5mL of Digestion Buffer.

### C. Generation of Fragments

1. Dissolve up to 10mg of pure lyophilized IgG in 1.0mL of Digestion Buffer, or add 0.5mL of the prepared sample to 0.5mL of Digestion Buffer.
2. Add the 1.0mL of sample to the tube or vessel containing the Immobilized Papain.
3. Incubate for five hours to overnight in a shaker water bath at 37°C at high speed. Maintain constant mixing of gel during the incubation. For human IgG, incubate for 4 hours using the same conditions.
4. If separating the immobilized enzyme from the digest by centrifugation, add 1.5mL of 10mM Tris•HCl, pH 7.5 to the digest before centrifugation, then remove the supernatant, which contains the IgG fragments. If using a resin separator, separate the digest from the immobilized enzyme, then wash the enzyme with the same Tris buffer described above and combine wash with digest.
5. The Fab fragments can be separated from undigested IgG and Fc fragments using an Immobilized Protein A column (Product No. 20356) or ion exchange chromatography.

## Additional Information

There are several enzymes that have been used in the fragmentation of immunoglobulins including pepsin, papain, trypsin, elastase, bromelain and ficin. Ficin is used to generate F(ab')<sub>2</sub> and Fab fragments from mouse IgG<sub>1</sub>, which is typically resistant to other digestion enzymes. Pepsin is most commonly used for the generation of F(ab')<sub>2</sub> fragments, and papain, although usually is used to generate Fab fragments, reports indicate that it also can be used to generate F(ab')<sub>2</sub> fragments.<sup>4</sup> To prepare F(ab')<sub>2</sub> fragments, the papain is first activated with 10 mM cysteine, which is then removed via gel filtration. If no cysteine is present during the papain digestion, F(ab')<sub>2</sub> fragments can be generated. These fragments are often inconsistent and reproducibility is a problem. If the cysteine is not completely removed, overdigestion may result.<sup>4</sup>

When using papain to generate Fab fragments, some species of IgG, such as rabbit, are difficult to digest and require a high ratio of enzyme to substrate. Other species of IgG, such as rat, are easily denatured. Digestion of various IgG species with non-immobilized and Immobilized Papain is reported in the literature (Table 1).

**Table 1.** Digestion of various IgG species with non-immobilized and Immobilized Papain as reported in the literature.

Species IgG	Digestion Method	Purification Method	Reference
<b>Human</b>			
Monoclonal IgG <sub>1</sub>	Papain	DEAE Cellulose	5
Polyclonal IgG	Crystalline Papain, Papain	DEAE Cellulose, Protein A	6, 7, 8
Anti- <i>Schistosoma mansoni</i> soluble egg antigens	Immobilized Papain	Immobilized Antigen	9
Cohn fraction II	Mercuripapain	DEAE	10
<b>Mouse</b>			
IgG <sub>2a</sub> from myeloma	Papain	Protein A	11
Polyclonal IgG	Crystalline Papain, Papain, Mercuripapain	DEAE, Protein A	6, 4
<b>Rat</b>			
Monoclonal IgG <sub>1</sub> , IgG <sub>2a</sub> , IgG <sub>2b</sub>	Mercuripapain	DEAE	2
Polyclonal IgG	Papain	DEAE	6
Polyclonal IgG subclasses	Papain	DEAE Cellulose or Protein A	4
IgG subclasses	Papain or Mercuripapain	Electrophoresis used to monitor digestion	12
<b>Rabbit</b>			
Polyclonal IgG	Immobilized Papain, Crystalline Papain, Mercuripapain	Protein A, CM Cellulose	3, 6, 4
<b>Sheep</b>			
Antidigoxin	Crystalline Papain	gel filtration, affinity chromatography	13
Polyclonal IgG	Crystalline Papain	DEAE Cellulose	6, 4
<b>Goat</b>			
	Crystalline Papain, Papain, Mercuripapain	DEAE, Protein A, ion exchange	6, 4
<b>Horse, Cow, Guinea Pig, Chicken</b>			
	Crystalline Papain	DEAE	6

## Related Thermo Scientific Products

44889	Cysteine•HCl, 5g
69710	Pierce® Resin Separators, 25/pkg
66385	Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 0.1-0.5mL
66528	Slide-A-Lyzer Concentrating Solution, 200mL
20356	Pierce Protein A Columns, 5 × 1mL settled gel pre-packed columns
44885	Fab Preparation Kit
44888	F(ab') <sub>2</sub> Preparation Kit
44880	IgG <sub>1</sub> Fab and F(ab') <sub>2</sub> Preparation Kit
25200-44	Precise™ Protein Gels (see catalog or web site for a complete listing)

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## General References

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11. Goding, J.W. (1978). Use of staphylococcal protein A as an immunological reagent. *J Immunol Meth* **20**:241-53.
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