



**Title:** *Digesting Antibodies with Pepsin to Yield F(ab)<sub>2</sub> Antibody Fragments*

No: RTLP-GL-Ab-3

Location:  
*Old CCRC Tripp Lab*

Approval Date:  
10 September 2004

Supersedes Date:

**Materials:**

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- |                                  |                             |                                      |                  |
|----------------------------------|-----------------------------|--------------------------------------|------------------|
| •Lab coat                        | •Equilibrated Protein       | •3 M Tris; pH 8.8                    | •Pipettes        |
| •Gloves                          | A-sepharose column          | •dH <sub>2</sub> O                   | •Pipetteman      |
| •100mM sodium citrate; pH 3.5    | •Pepsin                     | •Centrifuge                          | •Pipette Aid     |
| •Phosphate Buffered Saline (PBS) | •15 mL tubes                | •5 mL tubes                          | •Pipetteman tips |
|                                  | •37°C H <sub>2</sub> O bath | •Dialysis Tubing and Dialysis Clamps | •1L Beaker       |

**Overview:**

Human, rabbit and mouse IgG1 antibodies are resistant to cleavage at secondary sites within the F(ab)<sub>2</sub>. The resistance of murine Ig subclasses to pepsin-induced secondary digestion are: IgG1>IgG2a>IgG3>IgG2b.

**Procedure:**

1. Prepare a solution of IgG\* ranging between 2-5 mg/ml in 100-mM sodium citrate (pH 3.5). It is often best to purify the Ab of interest using a Protein A-sepharose column, as elution of Ab can be performed using the same 100 mM sodium citrate (pH 3.5) solution.

*\*NOTE: For mouse IgG2b, use elastase instead of pepsin to yield F(ab)<sub>2</sub> fragments. Replace the 100-mM sodium citrate buffer with Tris buffer (pH 8.8).*

2. Add 5 g of pepsin for each mg of Ab to be digested. Porcine pepsin with at least 3000 units of activity per mg is recommended. This procedure is best carried out in a 15 mL centrifuge tube.
3. Mix well and place in 37°C water overnight or at least 12h.
4. Stop reaction by adding a 1:10 volume of 3 M Tris (pH 8.8).
5. Centrifuge at 10,000 g for 30 minutes at 10°C.

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*NOTE: Cumulative g's may also be used if high speed centrifuge is not available.*

6. Separate F(ab)'<sub>2</sub> by running supernatant through a Protein A-sepharose column as described by the manufacturer.
7. The eluate is collected and saved, as this contains the F(ab)'<sub>2</sub> fragments.
8. Dialyze the eluate against 2 changes of PBS.

To verify digestion via SDS-PAGE: Non-reduced IgG migrates at 150 kD, non-reduced F(ab)'<sub>2</sub> run at 110 kD, non-reduced Fc migrates to 25 kD. Under reducing conditions, IgG yields 2 bands of 50 kD, and 25 kD. F(ab)'<sub>2</sub> yields a doublet at 25 kD and Fc on band at 25 kD.

Author	Management Approval/Date	Effective Date