

## FragIT - Column Solution for Fragmentation of Larger Quantities of IgG

### Introduction

Fab and F(ab')<sub>2</sub> antibody fragments are used in many applications where the presence of the Fc region of the antibody interferes. Fabricator is a unique proteolytic enzyme which cleaves IgG at a specific site below the hinge region. Pure Fab and Fc fragments are obtained within minutes.

Fragmentation of large amounts of IgG is typically associated with low yield, time consuming optimization and operation at low pH with loss of immunoreactivity as a consequence. Genovis has developed the FragIT self-drained column for fragmentation of larger quantities of IgG. The column contains Fabricator enzyme conjugated to NHS-activated agarose beads. As the IgG passes through the column it will be cleaved into pure F(ab')<sub>2</sub> and Fc fragments by Fabricator (Figure 1). The FragIT column is quick and precise with antibody cleavage under mild conditions and with high yield. The column is operated by gravity flow at room temperature. Furthermore, with the FragIT column there is no need to remove the Fabricator enzyme after fragmentation.

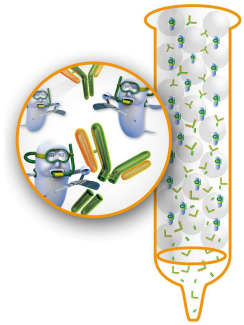


Figure 1. Schematic cleavage of IgG on FragIT column. IgG is cleaved by Fabricator to F(ab')<sub>2</sub> and Fc fragments as it runs through the column.

### Materials and Methods

The FragIT column was equilibrated with cleavage buffer (50 mM sodium phosphate, 150 mM NaCl, pH6.6). 20 mg Herceptin® (humanized IgG) at a concentration of 20 mg/ml was loaded on the column. The IgG was allowed to pass through the column using gravity flow. The flow rate was approximately 0.1 ml/min. Pure F(ab')<sub>2</sub> and Fc fragments were collected. The column was operated at room temperature. The eluted fragments were analyzed using SDS-Page under non-reducing conditions.

### Results and Conclusions

IgG was loaded on top of the FragIT column and as it passed through the column by gravity flow it was fragmented. F(ab')<sub>2</sub> (100kDa) and Fc (25 kDa) fragments were collected as illustrated in Figure 1. IgG was loaded at a concentration of 20 mg/ml. The digested fragments were analyzed with SDS-Page (Figure 2). More than 95% of the loaded IgG was cleaved to yield pure F(ab')<sub>2</sub> and Fc fragments and there was no over digestion.

The FragIT column is a rapid solution for cleavage of larger amounts of IgG. 20 mg IgG was run through the column and it was completely digested within 30 minutes after application.

With the FragIT column there is no need to remove the enzyme after fragmentation. (Fab')<sub>2</sub> and Fc fragments can be separated with (preparative) size exclusion chromatography.

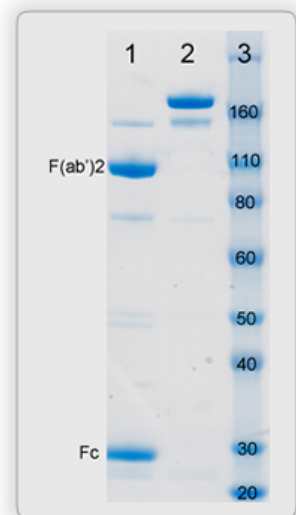


Figure 2. SDS-Page image of IgG cleaved by Fabricator in FragIT column. Lane 1: IgG (Herceptin®) run through FragIT column, Lane 2: IgG (Herceptin®) loaded on column (non-cleaved) and Lane 3: Molecular weight marker (Novex Sharp pre-stained marker, Invitrogen).