

FabRICATOR[®] - perfect F(ab')₂ fragments in minutes

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Introduction

Fab and F(ab')₂ antibody fragments are used in applications or assays where the presence of the Fc region may cause problems. If antibodies are used for staining of a specific target in tissues like spleen, lymph or in peripheral blood preparations, cells with Fc receptors (macrophages, monocytes, natural killer cells and B lymphocytes) can bind to the Fc region of the antibody and cause a background staining in areas that do not contain any antigen (1). Fab and F(ab')₂ fragments are also desirable for staining or binding to cell preparations in the presence of plasma since they can not bind to complement, which would lyse the cells (2). The divalency of the F(ab')₂ fragment enables it to cross-link antigens and hence makes it useful for rosetting, cellular aggregation and precipitation assays (3).

F(ab')₂ fragments are generally produced using pepsin. Here a novel method for antibody fragmentation using the enzyme FabRICATOR[®] is introduced. This enzyme is a partially modified and his-tagged IdeS enzyme (4). FabRICATOR[®] cleaves IgG in the hinge region leaving an intact F(ab')₂ and two residual Fc fragments.

Materials and Methods

Generation of F(ab')₂ fragments – digestion of IgG

1 mg of polyclonal human IgG (including all subclasses) was digested with 0,1 nmol FabRICATOR[®] for 30 minutes in 0.1 M Sodium phosphate buffer pH 6.6, 150 mM NaCl at 37 °C. Samples were withdrawn after 5,15 and 30 minutes for analysis on SDS Page gel electrophoresis.

Purification of antibody fragments

Particles carrying protein A and magnetic particles carrying chelated nickel ions (both particles are under development for the FabRICATOR[®] kit) were added to the digested IgG in order to remove Fc fragments and FabRICATOR[®]. The particles were added subsequently to make it possible to analyze each separation step separately. Both separation steps were carried out at room

temperature for 30 minutes and then the particles were collected with a lab magnet.

Sample from both separation procedures were collected for analysis on SDS Page gel electrophoresis.

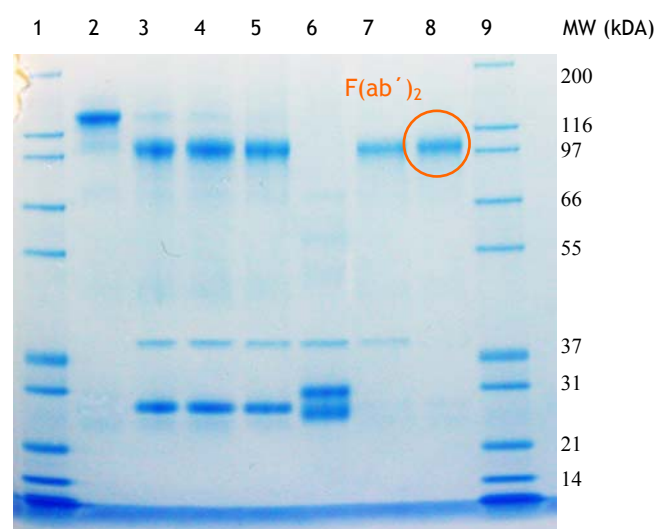


Figure 1: SDS Page

Lane 1 Molecular weight marker Mark 12™ (Invitrogen)

Lane 2 IgG fraction

Lane 3 5 min digestion with FabRICATOR[®]

Lane 4 15 min digestion with FabRICATOR[®]

Lane 5 30 min digestion with FabRICATOR[®]

Lane 6 30 min digestion with FabRICATOR[®] (*reduced sample*)

Lane 7 After removal of Fc fragments

Lane 8 After removal of FabRICATOR[®]

Lane 9 Molecular weight marker Mark 12™

Analysis on SDS PAGE gel

Cleavage products were analyzed by SDS –PAGE in 4 to 12% gradient gels under both reducing and non-reducing conditions. Gels were stained using Simply Blue™ Safe Stain (Invitrogen) according to protocol recommended by the supplier.

Results and Discussion

All four subclasses are readily cleaved after 30 minutes of incubation with FabRICATOR[®] and already after 5 minutes, the digestion is almost

complete. The 150 kDa IgG molecule is cleaved into one larger fragment of approximately 100 kDa and one smaller fragment of 25 kDa (the Fc region of the antibody). Upon reduction of the disulfide bridges, the 100 kDa fragment is visible as two bands on the SDS Page gel, one 31 kDa band and the light chain of 24 kDa.

After treatment with protein A and Ni chelating magnetic particles the Fc parts and FabRICATOR[®] have been successfully removed from the reaction tube leaving the resulting pure F(ab')₂ fragment in the supernatant.

Conclusions

FabRICATOR[®] has unique proteolytic properties and hence serves as an excellent tool for antibody fragmentation. The yield of the reaction is high and the enzyme does not further digest the resulting products. In combination with magnetic particles it is possible to produce pure F(ab')₂ fragments using FabRICATOR[®] in less than 60 minutes with an easy and very user friendly process.

References

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