

Antibody fragmentation of F(ab)₂' and Fc for epitope activation diagnostics

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Antibody fragment such as F(ab)₂' can be advantageous of its reduced steric hindrance, reduced non-specific binding, lower immunogenicity. FabRICATOR®, an IdeS, is a commercially available cysteine proteinase (from Genovis, Inc., Sweden) that can specifically cleave the C-terminus end of the hinge region of IgG. We evaluated the FabRICATOR's cleavage performance and devised the subsequent recovery process to obtain purified forms of Fc and F(ab)₂'. It was demonstrated by SDS-PAGE analysis that FabRICATOR was able to completely cleave both polyclonal and monoclonal antibody molecules under the same condition. The process to recover purified forms of Fc and F(ab)₂' consisted of three steps. SDS-PAGE analyses indicated the isolation process was effective and successful. Activity of the F(ab)₂' of the anti-HBsAg (hepatitis B surface antigen) was analyzed by SPR to compare with that of the native anti-HBsAg. Since Fc is known to prolong the retention time in vivo, we attempted to conjugate the Fc fragment to epidermal growth factor (EGF) by amine coupling. We expect that the F(ab)₂' and Fc show superior binding characteristics in vitro diagnostics and enhance a protein's in vivo serum stability, respectively.