open daccess y protocols.io

Merits You Should Know about Immunoglobulin F(ab) and F(ab')2 fragments

bello smitu

Abstract

Overview of the fragment antigen binding

The fragment antigen binding (Fab fragment) is an antibody structure which still binds to antigens although it is monovalent with no Fc portion. Antibody that is digested by the enzyme papain will yields two fab fragments of about 50 kDa each and an Fc fragment.

However, fab2 fragment antibodies are produced by pepsin digestion of whole IgG antibodies in order to remove the Fc region while leaving intact some of the hinge region. They have two antigen-binding fab portions linked together by disulfide bionds and then are divalent with a molecular weight of about 110kDa.

The advantages of fragment secondary fab antibodies

- 1. F(ab) and F(ab')2 fragment antibodies eliminate non-specific binding between Fc portions of antibodies and Fc receptors on cells.
- 2. Penetrate tissues more efficiently due to their smaller size.
- 3. Since fragment antibodies do not have Fc portions, they do not interfere with anti-Fc mediated antibody detection.

Reasons for choosing a <u>fab fragment</u>

- 1. Monovalent antibody fragments (F(ab) fragments) are powerful tools to block background from primary antibody binding and in double staining experiments.
- 2. F(ab) fragments are used to block endogenous immunoglobulins on cells, tissues and exposed immunoglobulins in multiple labeling experiments using primary antibodies from the same species.
- 3. After the blocking step with normal serum, we recommend incubating F(ab) fragments in excess to block endogenous immunoglobulins in IHC. These antibodies are not recommended for blocking immunoglobulins in WB and ELISA.

Reasons for choosing a F(ab')2 fragment

1. Divalent antibody fragments (F(ab')2 fragments) are smaller than whole IgG molecules and enable a better penetration into tissue thus faciliting better antigen recognition in IHC. The

- use of F(ab')2 fragments also avoids unspecific binding to Fc receptor on live cells or to Protein A/G.
- 2. F(ab')2 fragments are not recommended for blocking since they have two binding sites that are available to capture the primary antibody introduced subsequently. However, as opposed to F(ab) fragments, F(ab')2 fragments can both bind and precipitate antigens thanks to their two binding sites. We recommend using normal serum with these antibodies to prevent the binding to Fc receptors.

Citation: bello smitu Merits You Should Know about Immunoglobulin F(ab) and F(ab')2 fragments. **protocols.io**

dx.doi.org/10.17504/protocols.io.s2hegb6

Published: 28 Aug 2018

Protocol