

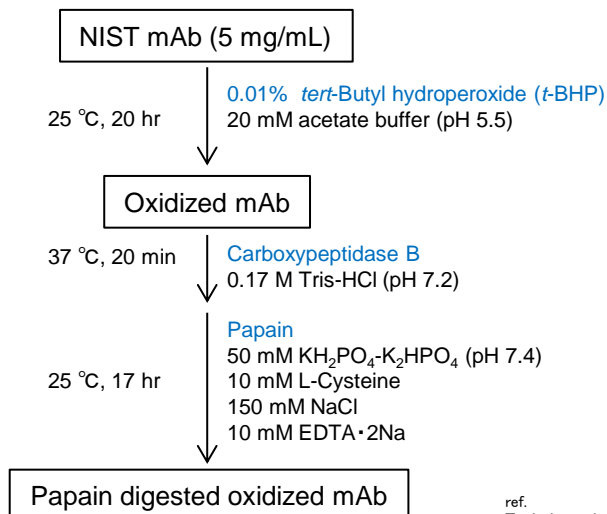
## Analysis of oxidized monoclonal antibodies by hydrophobic interaction chromatography

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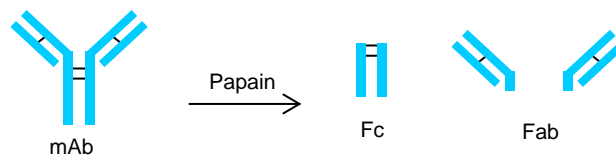
During manufacture and/or storage of biopharmaceuticals, variants with different properties from desired substances are produced by enzyme reactions or physicochemical interactions. Characterization of the variants is of great importance from the perspective of ensuring efficacy and safety of pharmaceutical products.

Oxidized mAb variants can be analyzed by hydrophobic interaction chromatography (HIC). In this report, we introduce the separation of mAb samples and their oxidized species using our HIC column, BioPro HIC BF.

### mAb oxidation with *t*-BHP treatment

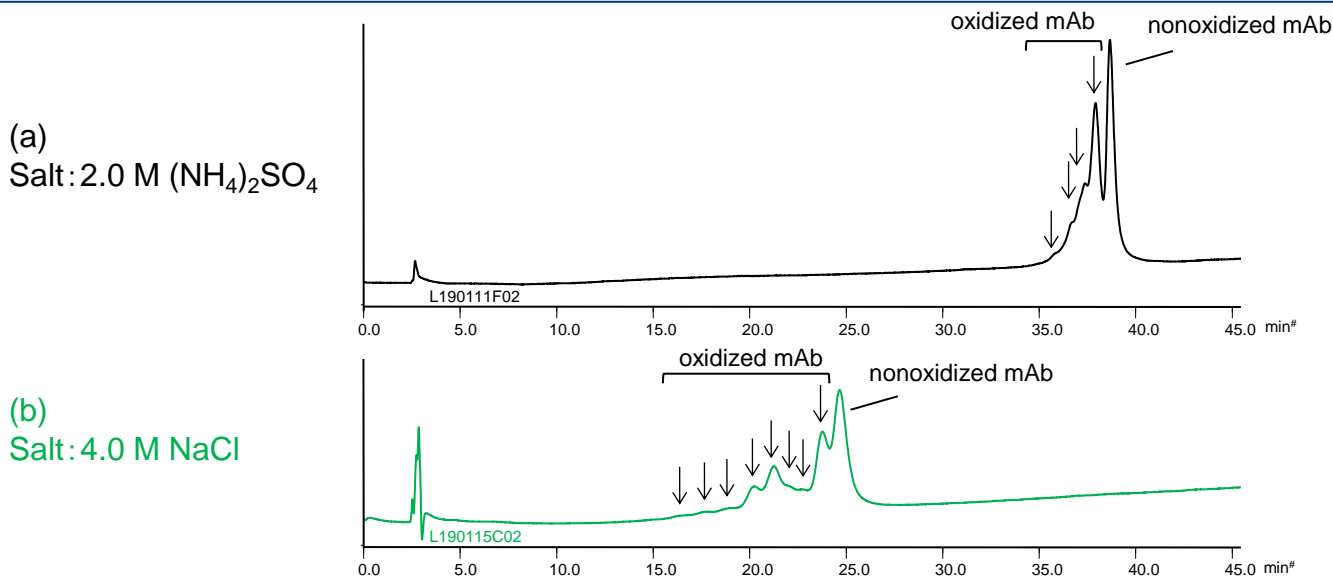


*tert*-Butyl hydroperoxide (*t*-BHP) was used as a chemical oxidant to promote oxidation of methionine residues of NIST mAb. Subsequently, papain was used for the preparation of two Fab fragments and one Fc fragment.



ref. Techniques in Protein Chemistry Volume 7, 1996, Pages 275-284

### Analysis of oxidized mAb



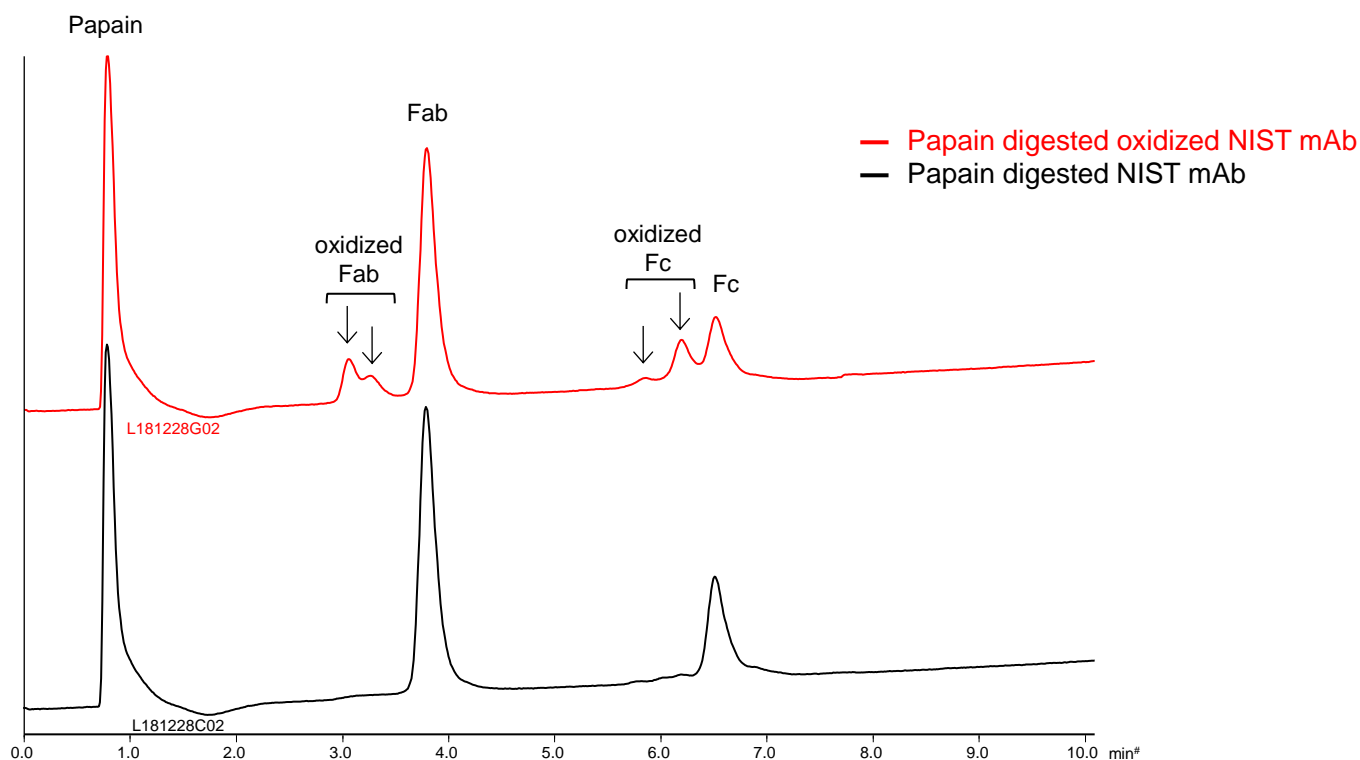
Column : BioPro HIC BF 4 μm, 100 X 4.6 mm I.D.  
 Eluent : A) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing salt  
 B) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0)  
 40-80%B (0-40 min), 80%B (40-45 min)  
 Flow rate : 0.3 mL/min  
 Temperature : 25°C  
 Detection : UV at 280 nm  
 Injection : 5 μL (1.0 mg/mL)

BioPro HIC BF column demonstrated the separation of oxidized mAb from the nonoxidized mAb using a low flow rate and shallow gradient slope.

In the chromatogram (a), earlier eluting four peaks were assumed to be derived from species that would have oxidized methionine residues on the mAb. The oxidation of sulfide side chains on methionine residues might result in conformational changes.

By using sodium chloride instead of ammonium sulfate, better resolution was achieved with a short analysis time (b).

## Analysis of papain digested oxidized mAb



Column : BioPro HIC BF 4  $\mu$ m, 100 X 4.6 mm.I.D.  
Eluent : A) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0)  
containing 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
B) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0)  
40-80%B (0-10 min)  
Flow rate : 1.0 mL/min  
Temperature : 25°C  
Detection : UV at 280 nm  
Injection : 5  $\mu$ L (0.5 mg/mL)

Papain digested NIST mAb samples were analyzed. Fab and Fc peaks were observed on analysis of the nonoxidized mAb sample. Peaks that would correspond to oxidized Fab and Fc were observed on the analysis of papain digested oxidized mAb sample. The oxidized species eluted earlier than the nonoxidized species.

\*Journal of Chromatography A, 2008, 1214, 81-89