HPLC DATA SHEET

Analysis of oxidized monoclonal antibodies by hydrophobic interaction chromatography

U190422AE

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

NIST mAb (5 mg/mL)

25 ℃, 20 hr — 0.01% *tert*-Butyl hydroperoxide (*t*-BHP), 20 mM acetate buffer (pH 5.5)

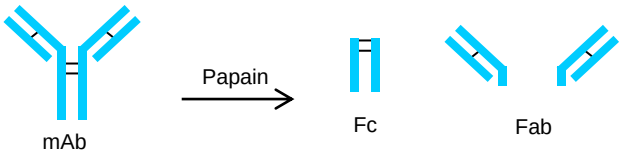
Oxidized mAb

37 ℃, 20 min — Carboxypeptidase B, 0.17 M Tris-HCl (pH 7.2)

25 ℃, 17 hr — Papain, 50 mM KH_2PO_4-K_2HPO_4 (pH 7.4), 10 mM L-Cysteine, 150 mM NaCl, 10 mM EDTA・2Na

Papain digested oxidized mAb

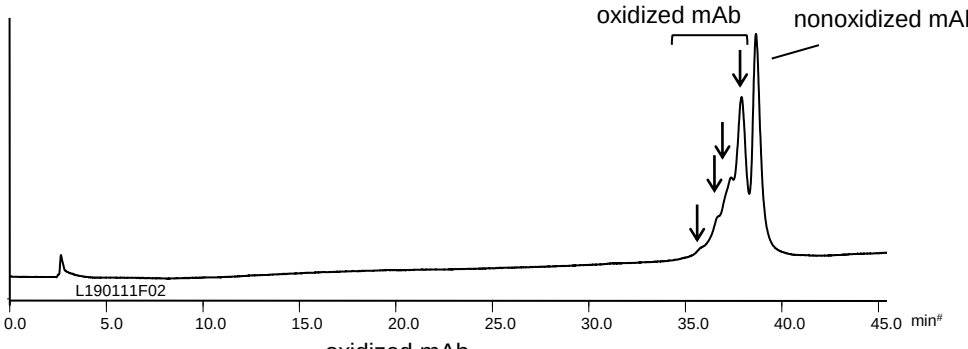
tert-Butyl hydroperoxide (*t*-BHP) was used as a chemical oxidant to promote oxidation of methionine residues of NIST mAb.
Subsequently, papain was used for the preparation of two Fab fragments and one Fc fragment.



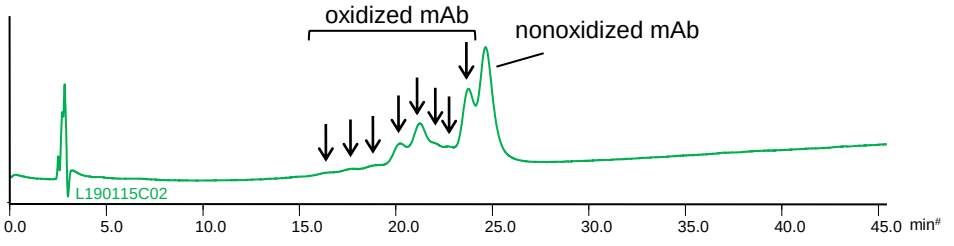
ref.
Techniques in Protein Chemistry Volume 7, 1996, Pages 275-284

Analysis of oxidized mAb

(a)
Salt : 2.0 M $(NH_4)_2SO_4$



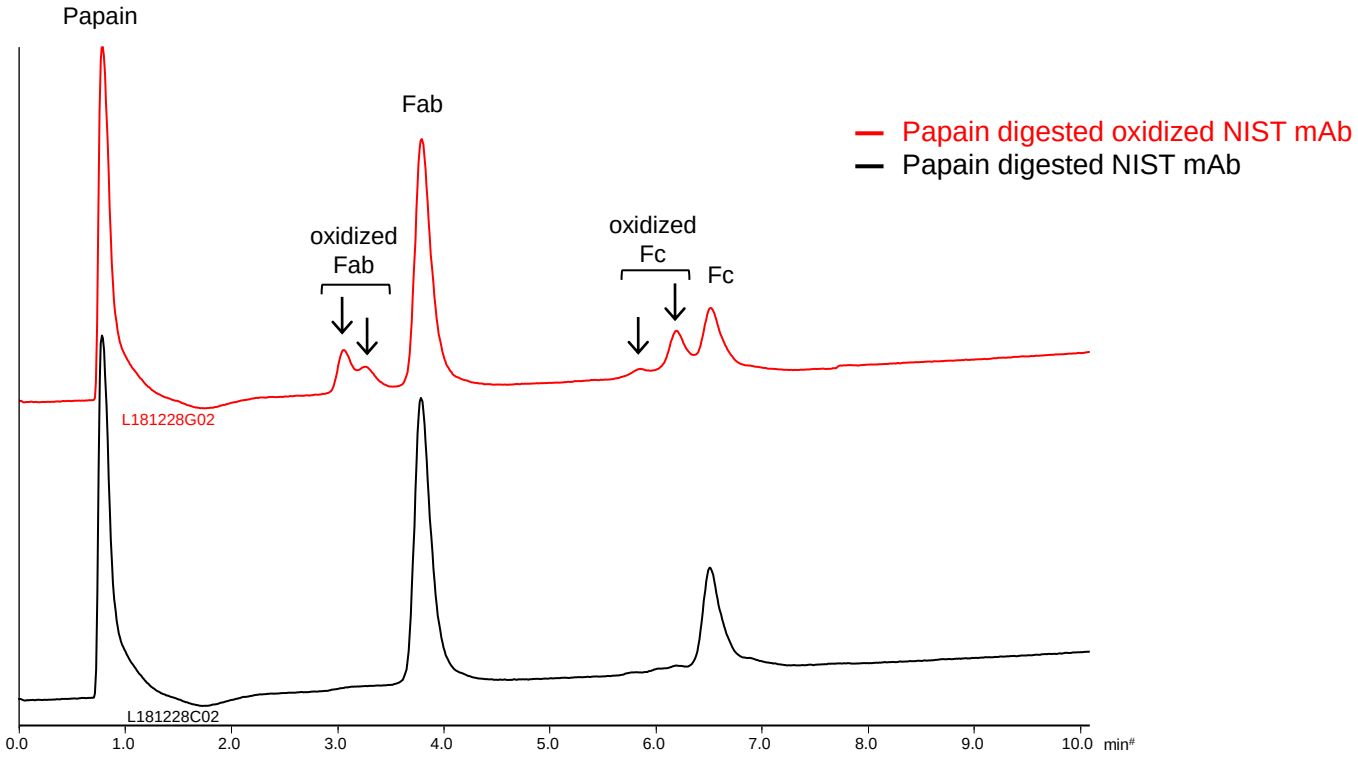
(b)
Salt : 4.0 M NaCl



Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt
	B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
	40-80%B (0-40 min), 80%B (40-45 miin)
Flow rate	: 0.3 mL/min
Temperature	: 25℃
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 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
	B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
	40-80%B (0-10 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 5 µL (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