Ab-Capcher ExTra™ and Protein L Purification of human IgG Fab fragment

IgG papain digestion

Undigested IgG and Fc fragment removal

Recovery of Fab fragment

Reaction protocol

Human IgG (10 mg/mL)

↓ Add 1/10 volume of papain (1 mg/mL) and react at 37° C for 1 hour

↓ Add 1M iodoacetamide (final 15mM) to stop the reaction

Ab-Capcher ExTra™ packed column (equilibrated with PBS)

↓ Add papain digest to the column

↓ Collect the raw material (FT) fraction

Protenova Protein L-immobilized gel packing column (equilibrated with PBS)

↓ Ab-Capcher ExTra™ FT fraction added to the column

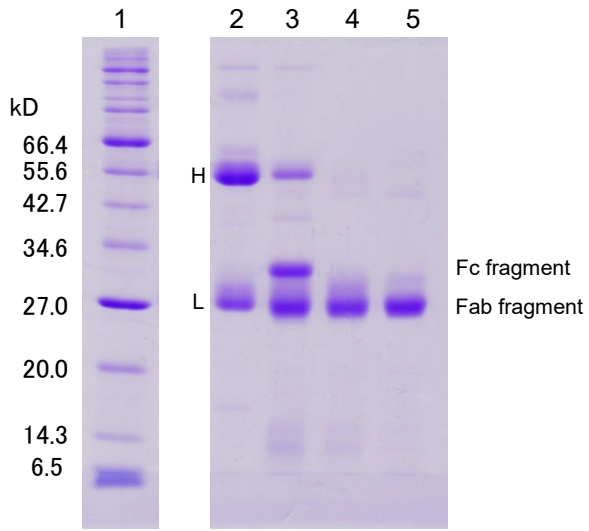
↓ FT fraction collection

↓ Wash the column with PBS

↓ Elute with 0.1M glycine-HCl pH 2.5

↓ Neutralize the elution fraction with 1M Tris

Purification completed



SDS-PAGE
Gel: 15% a.a. (Tris-Glycine)
Stain: CBB

Lane 1: MW marker
Lane 2: human IgG
Lane 3: human IgG after papain digestion
Lane 4: Ab-Capcher ExTra™ FT fraction
Lane 5: Protein L elution fraction

Fab fragments were purified from human IgG using Ab-Capcher ExTra™ and Protenova Protein L-immobilized gel. Papain digestion reduced human IgG into Fab and Fc fragments (lane 3). By adding this papain digest to an Ab-Capcher ExTra™ packed column, undigested IgG and Fc fragment were adsorbed to the column, and the FT fraction containing Fab was collected (lane 4). This FT fraction was added to a Protenova Protein L packed column, and the eluted fraction was collected. SDS-PAGE confirmed that high purity Fab fragments were obtained in the protein L column elution fraction (lane 5). Thus, it was found that the combination of Ab-Capcher ExTra™ and Protenova Protein L-immobilized gel was effective for purification of Fab fragments.

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