HPLC DATA SHEET

High resolution analysis of monoclonal antibodies and fragments using YMC-Pack Diol

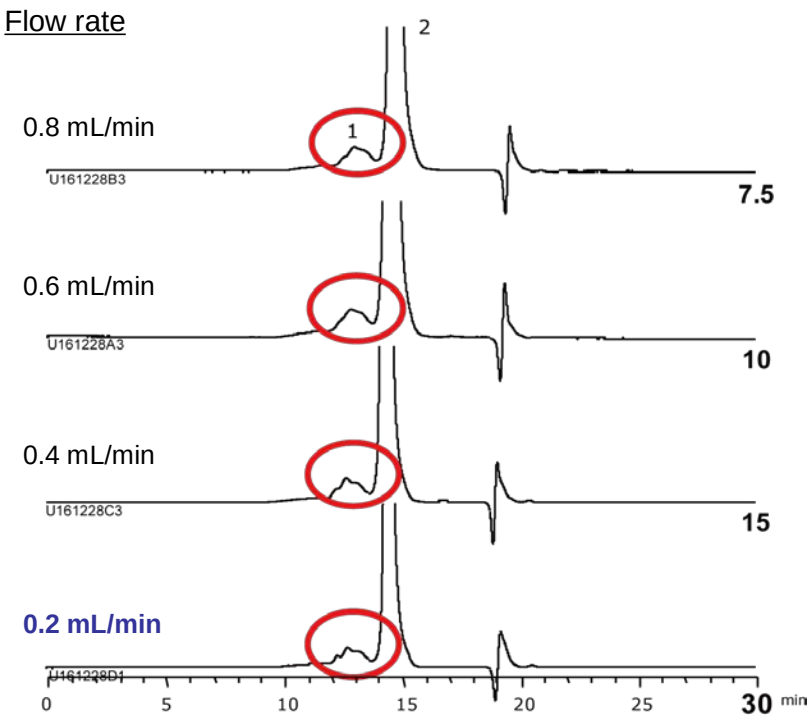
R170831AE

YMC-Pack Diol is a silica gel based size exclusion chromatography (SEC) phase which comes in range of particle sizes and pore sizes for the optimum separation of different biomolecules. Columns packed with 2 µm particles are effective for rapid analysis and high resolution of aggregates and fragments of biopharmaceuticals.

Analysis of monoclonal antibody and its aggregates

● Influence of flow rate on antibody analysis

YMC-Pack Diol-300, 2 µm, 300 X 4.6 mmI.D.

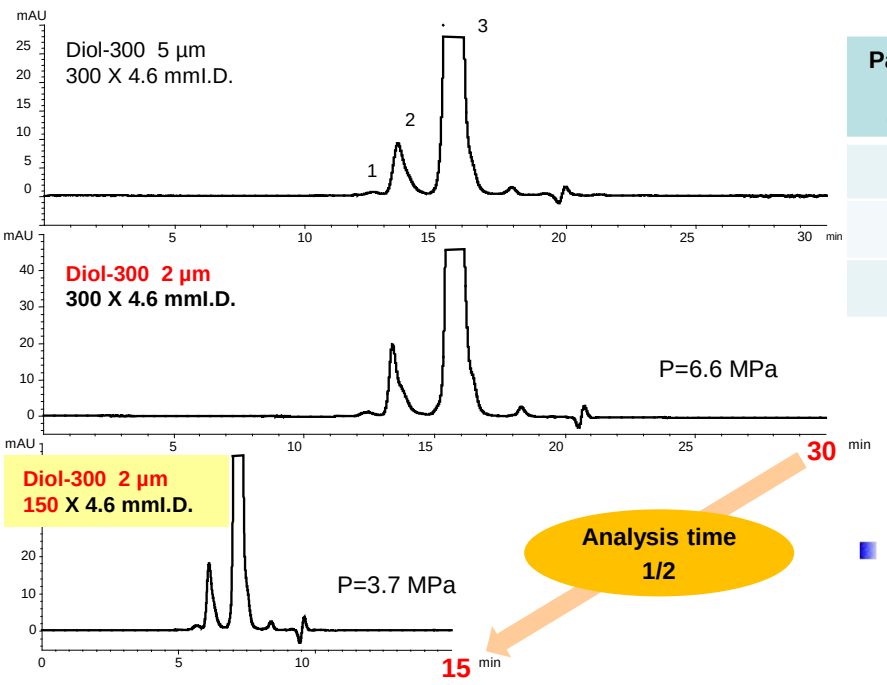


Flow rate (mL/min)	N (2)	P (MPa)
0.8	7,300	28.3
0.6	8,900	20.9
0.4	9,400	14.4
0.2	15,200	7.2

Column	: YMC-Pack Diol-300, 2 µm, 300 X 4.6 mmI.D.
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Detection	: UV at 280 nm
Temperature	: ambient
Sample	: Humanized monoclonal IgG1

- Diol-300 2 µm column was used for monoclonal antibody analysis. Resolution between aggregates and monomer is improved as the flow rate is decreased.
- Higher flow rates are suitable for increased sample throughput, especially when the resolution is sufficient.

● Increasing throughput by using the 2 µm column

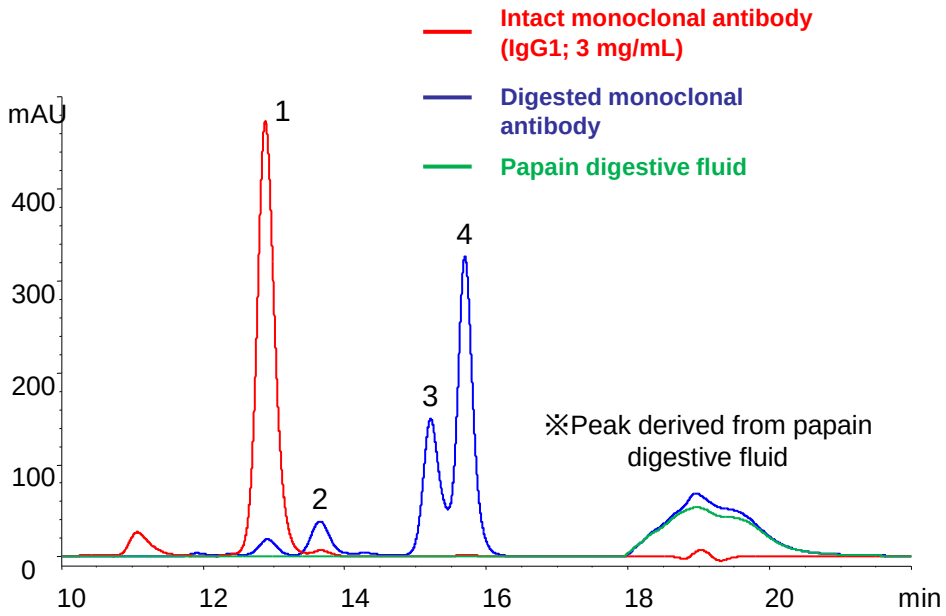


Particle size (µm)	Column size length X I.D. (mm)	N (3)	Rs (1,2)	Rs (2,3)
5	300 X 4.6	8,500	0.88	2.67
2	300 X 4.6	16,200	1.17	4.15
2	**150 X 4.6**	8,700	0.85	2.75

Column	: YMC-Pack Diol-300, 2 µm,
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Detection	: UV at 280 nm
Temperature	: ambient
Sample	: Humanized monoclonal IgG1

- The 2 µm, 150 mm length column offers the same resolution as the 5 µm, 300 mm length column. This means the analysis time can be reduced by half by changing the particle size from 5 µm to 2 µm.

Analysis of monoclonal antibody fragments by using YMC-Pack Diol-200 column



Method of papain digestion

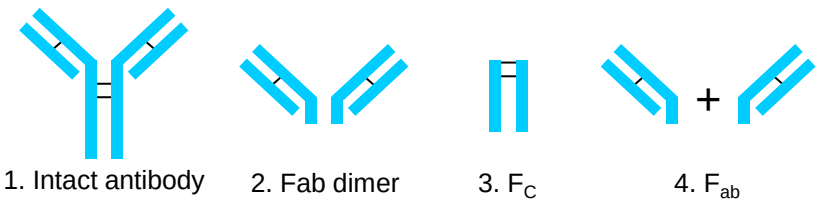
Buffer(pH 7.5)

Papain 0.1 g/L
NaCl 0.15 mol/L
L-cysteine 0.01 mol/L
EDTA 0.01 mol/L

Monoclonal antibody 3 mg/mL

25℃, 17.5 hours

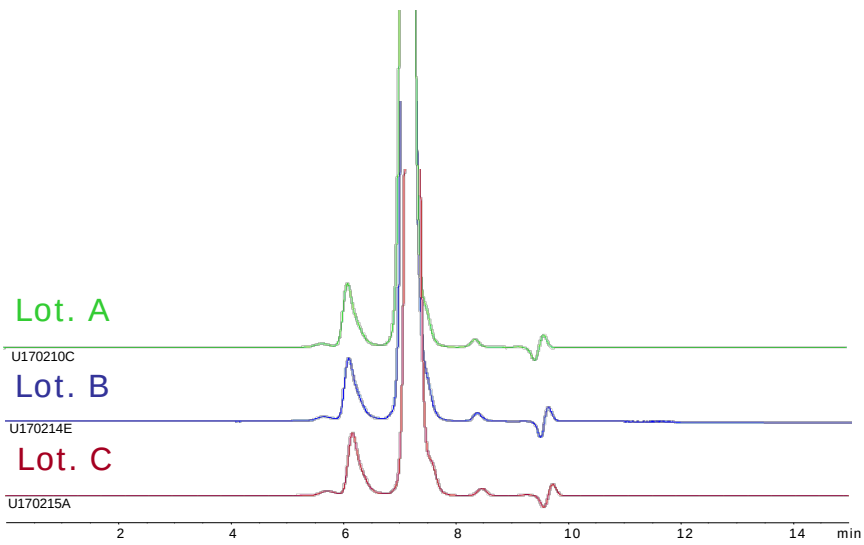
SEC analysis



Column	: YMC-Pack Diol-200, 2 µm 300 X 4.6 mmI.D.
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Detection	: UV at 280 nm
Temperature	: ambient

- A monoclonal antibody digested by papain, a proteolytic enzyme, as well as the intact monoclonal antibody were analyzed using a YMC-Pack Diol-200 column.
- By using a high resolution 2 µm column, intact antibody and fragments were well separated.

Lot-to-lot reproducibility of YMC-Pack Diol 2 µm



Column	: YMC-Pack Diol-300, 2 µm 150 X 4.6 mmI.D.
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Detection	: UV at 280 nm
Temperature	: 25℃
Sample	: Humanized monoclonal antibody

- YMC-Pack Diol 2 µm columns offer high lot-to-lot separation reproducibility. This feature greatly contributes to characterization of antibodies in areas of research as well as quality control of biopharmaceuticals.