# Rapid HPLC Analysis of Monoclonal Antibody $IgG_1$ Light Chains Using ZORBAX Poroshell 300SB-C8

## Application

**Biochemical**

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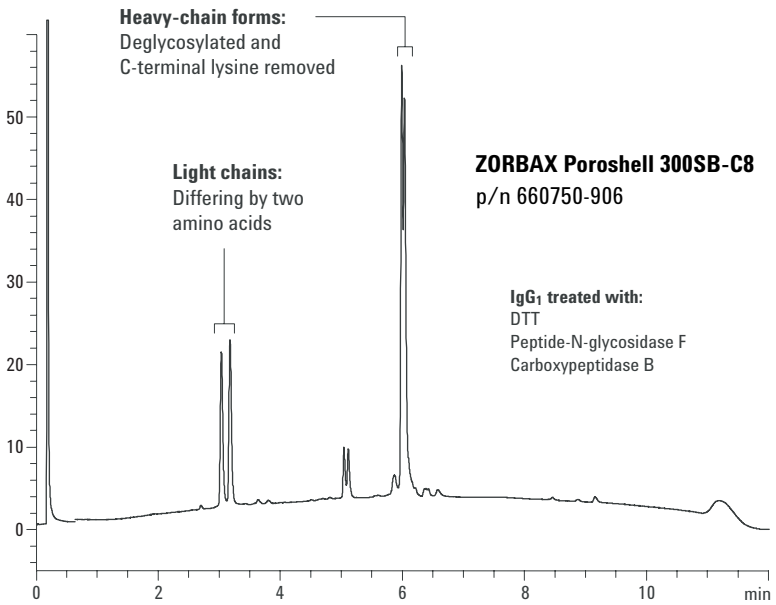
Antibodies represent a class of proteins that are the key to immunological interaction. They bind to an antigen (protein, glycoprotein, DNA, etc.) with extreme specificity. This makes antibodies extremely valuable for use in diagnostics, general research, and for therapeutics. IgG is a class of antibodies having two heavy (50 kDa each) and two light chains (25 kDa each), attached through disulfide bridges. While their carboxy-terminal domains are conserved, the amino-terminal domains are variable in amino-acid sequence; thus producing the molecule's specificity and diversity. Most antibodies are glycosylated, further increasing molecular diversity. Treatment of intact antibodies with various chemicals and enzymes allows the specific cleavage of the heavy and light chains, removal of carbohydrate moieties, and cleavage of the polypeptide chains. In this manner, one may specifically study the structure of a particular antibody. The rapid HPLC method development and analyses possible using ZORBAX Poroshell technology are valuable tools for this type of structural investigation of antibodies.




**Conditions**

| | |
|---|---|
| Column | **ZORBAX Poroshell 300SB-C8** (2.1 × 75 mm, 5 µm) p/n 660750-906 |
| Temperature | 70 °C |
| Flow rate | 1.0 mL/min |
| Detection | UV (210 nm) |

## Highlights

- High-velocity separations using ZORBAX Poroshell technology result in high-resolution analysis of antibodies with a short run time
- Method development is also more rapid, since these run times are shortened using ZORBAX Poroshell technology
- ZORBAX Poroshell 300SB columns come in many internal diameters and bonded phases. This gives a wide variety of choices for the optimal fast separation of proteins and peptides
- The choice of which ZORBAX Poroshell column to use will depend on the molecular weight (MW) and heterogeneity of the protein sample

**Mobile phase**

A = $H_2O$-ACN (90:10)

B = $H_2O$-ACN (10:90)

Both A and B contain 0.1% TFA and 3 mL/L of PEG 300.

**Gradient timetable**

| Time (min) | % Solvent B |
|---|---|
| 0.00 | 20.0 |
| 10.00 | 50.0 |
| 10.10 | 20.0 |
| 12.00 | 20.0 |

The chromatogram shows a rapid reversed-phase analysis optimized for the separation of antibody light chains (peaks eluting at 3 min). Two heavy-chain forms can also be observed, eluting closely together at 6 min. While resolution of the heavy chains is sacrificed, optimization of the gradient profile allows one to distinguish between light chains of 25 kDa differing only in the identity of two amino acids. This rapid, high-resolution analysis can be used to: quantitate the chain forms; follow additional enzymatic reactions and modifications; or isolate antibody chains for downstream use.

ZORBAX Poroshell technology facilitates rapid analysis and method development of large molecules by use of its shortened diffusion path and tolerance of high linear velocity (high relative flow rate). ZORBAX Poroshell particles consist of a solid silica core covered by a thin totally porous crust. Large molecules, which diffuse very slowly compared to small molecules, can move into and out of the thin crust in a very short time. Flow rate can be increased to reduce run time, without significant peak broadening. ZORBAX Poroshell columns are typically used at flow rates five to ten times those used with a column of the same dimensions containing totally porous particles. Note that the analysis is complete in less than 10 min. The resolution achieved is similar to that of a 50-min run on a column having totally porous particles. Finally, the short column length and 5-µm particle diameter function together to keep back pressure within acceptable limits.

Separations on ZORBAX Poroshell often benefit from increased flow rate because this increases gradient volume and reduces the gradient slope, resulting in increased relative retention (k') and resolution (Rs). The resolution of a 50-min run is achieved in 10 min! The additional benefit of using a rapid, high-resolution separation in method development should not be overlooked. A series of scouting runs may be made in, for example, one-fifth the usual time. This gives the analyst more time to achieve the type of resolution required for separation of light- (or heavy-) chain forms. For an example of a rapid ZORBAX Poroshell separation optimized for IgG heavy-chain analysis, see Agilent publication number 5989-0070EN.

## For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.
Search "Poroshell".

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