A044

HIGH-SPEED QUANTIFICATION OF IMMUNOGLOBULIN G
CIMac™ r-Protein A Analytical Column

CIMac™ r-Protein A Analytical Column is a short bed, high performance monolithic column. It is intended for fast, efficient, and reproducible qualitative and quantitative analyses of Immunoglobulin G (IgG) and suitable for use with HPLC and UPLC systems. Quantification of IgG is possible between 0.2 µg and 20 µg. Its small volume and short column length allow operation at high volumetric flow rates (up to 3 mL/min). The information about product quantity and purity is thus generated in just 1 minute! The column has an innovative symmetric design for bi-directional flow contributing to longer lifetime.

Chromatographic Analysis of IgG

Column:	CIMac™ r-Protein A Analytical Column (recombinant protein A produced in *E.coli* coupled to carboxyimidazole-activated monolith); Catalog number: 110.1004-2
UV detection:	UV at 280 nm
Mobile phases:	Buffer A: phosphate buffered saline; pH 7.2; Buffer B: 0.1 M glycine; pH 2

Concentration curve was obtained with polyclonal human IgG and was used as a reference for quantification of IgG in samples (Figure 1).

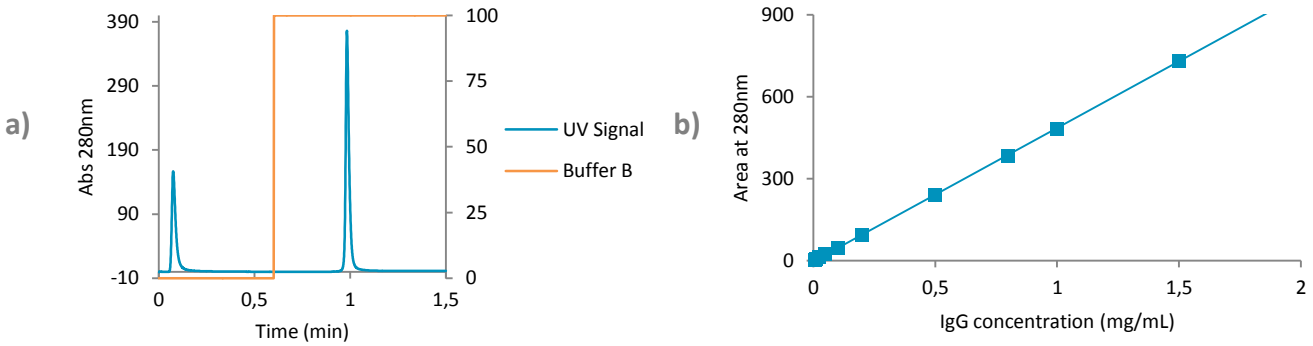
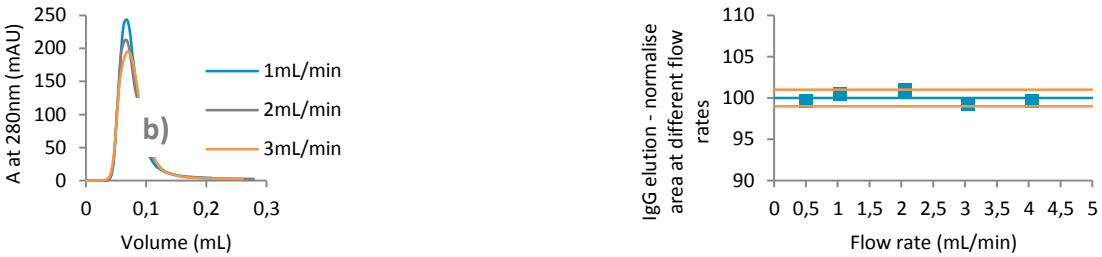


Figure 1: Quantification of IgG with **CIMac™ r-Protein A Analytical Column**. a) Representative chromatogram of IgG quantification and b) calibration curve of IgG. Agilent series 1200 HPLC System was used for chromatographic analysis. 10 µL of polyclonal human IgG was injected at indicated concentrations in presence of uracil in phosphate buffered saline, pH 7.2 at flow rate: 1.5 mL/min.

Quantification of human polyclonal IgG with CIMac™ r-Protein A Analytical Column

Column geometry and pore size enable efficient separation and quantification of IgG at extremly high flow rates. Tailing factor and elution volume for IgG are preserved with a flow rate increase, demonstrating convective mass transfer desorption mechanism. Elution volume for IgG is 0.15 mL, corresponding to only 1.5 column volumes (Figure 2a). Pressure drop is linear with flow rate increase as shown in Figure 3b. Less than 1% deviation in calculated peak areas in the flow rate interval between 0.5 and 4.0 mL/min is obtained (Figure 2b).



Flow distribution through the monolith from both directions

Figure 2: IgG quantification is flow independent. A) overlay of IgG elution curves at three flow rates and b) normalised area of elution peak at different flow rates. A low pressure gradient analytical Knauer Smartline HPLC was used. 20 µL of polyclonal human IgG (0.5 mg/mL) in phosphate buffered saline was injected at pH 7.2 at flow rates: 0.5-4.0 mL/min and eluted with 0.1 M glycine, pH 2.0.

CIMac Protein A housing geometry is designed to allow identical flow distribution through the column in both directions (Figure 3 a). Using the column in both directions at least doubles the life time of the column, as changing the flow direction during the IgG quantification simoultanesly serves as a column washing step.

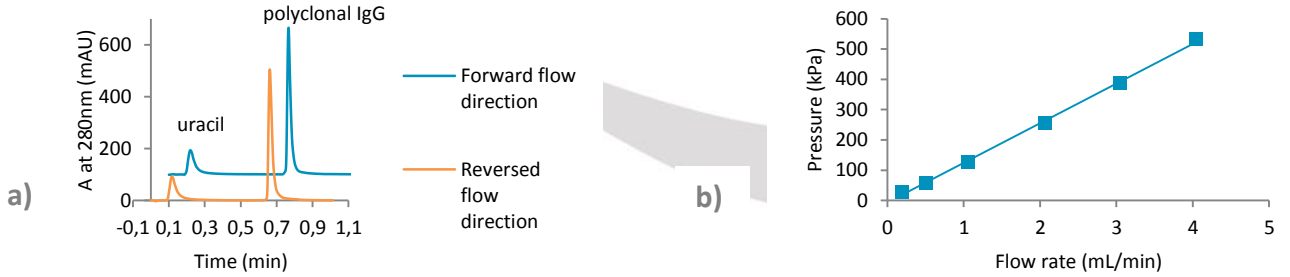


Figure 3: Bi-directional flow and linear dependence of pressure on the flow rate. A) Flow direction does not influence the quality of chromatographic separation. B) Relationship between flow rate and pressure drop over the column is linear. Analytical Knauer HPLC was used for analyses. 10 µL of polyclonal human IgG (1.0 mg/mL) and uracil (0.05 mg/mL) in phosphate buffered saline was injected at pH 7.2 at flow rate: 1.0 mL/min and eluted with 0.1 M glycine, pH 2.0.

Quantification of IgG in CHO supernatant

To demonstrate the performance of the column, an optimised chromatographic method for quantification of mAb in CHO supernatant was developed, where a chromatographic run was completed in 0.5 min (Figure 4a). Such speed of chromatography enables quantitative analysis of more than 100 samples per hour. The purity of elution fraction was confirmed by SDS-PAGE analysis under non reducing conditions (Figure 4b).

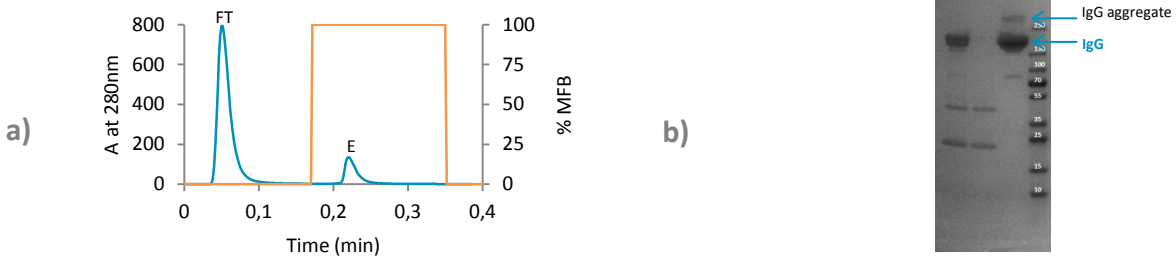


Figure 4: A) Quantification of IgG in CHO cell supernatant.Analytical Knauer HPLC was used. 10 µL of monoclonal antibody (mAb) in CHO supernatant, diluted 4-times in phosphate buffered saline, pH 7.2 was injected at flow rate 2.0 mL/min and eluted with 0.1 M glycine, pH 2.0. B) SDS-PAGE Analysis (L=load, FT=flow-through, E=elution, LMW=low molecular weight marker)

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