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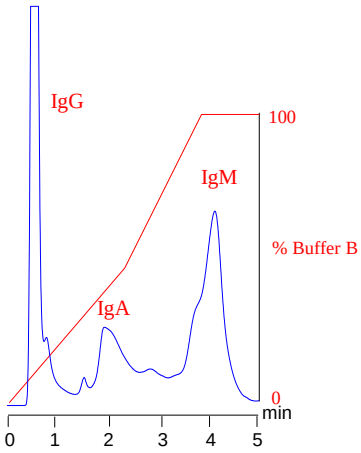
APPLICATION NOTE

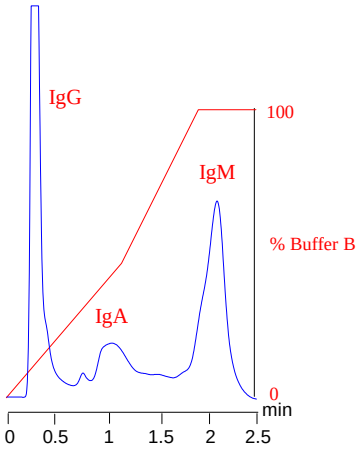
Fast Separation of IgG, IgA and IgM on STYROS™ DEAE/NB: *Narrow Bore Weak Anion Exchanger.*

The analysis of all proteins in a biological sample by 2-D PAGE is limited due to the high variation in protein abundance.
At low sample load, only the high-abundance or *housekeeping* proteins (more than 10,000 copies per cell) are detectable. If the load is increased to detect low abundance proteins (10 to 1,000 copies per cell), the gel is overloaded by high-abundance proteins, and the resolution is lost.
A prefractionation of the sample is needed to separate the components with high concentration or high abundance.
In the following example a **STYROS**™ DEAE Narrow Bore column is used to separate high concentration of IgG from IgA and IgM.



Chromatogram 1
STYROS™ DEAE/NB 2.1 X 150 mm
1 ml/ min (1,700 cm/hr).

The bulk of the IgG can be separated at the start of the run while the other two components exhibit higher retention and therefore elute with higher salt.
To be noted that the separation is done at linear flow rates of 1,700 cm/hr allowing short runs.
The run time is reduced in half in chromatogram 2.
The same separation is carried at 3,400 cm/hr of linear velocity during a 2.5 min period without affecting the overall resolution.
STYROS™'s performance at high speed and short run time provides the possibility to prefractionate samples in order to remove/reduce high concentration components interfering with the resolution, prior to running 2D gel electrophoresis.



Chromatogram 2
STYROS™ DEAE/NB 2.1 X 150 mm
2ml/ min (3,400 cm/hr) separation.

Table 1. Operating parameters for the chromatograms.

HPLC System.	HP 1100
Column	**STYROS**™ DEAE/NB 2.1 X 150 mm
Mobile Phase	A: 20 mM Phosphate, pH =7 B: A + 1 M NaCl, pH = 7
Flow rate	1 and 2 ml/min (1,700 cm/hr and 3,400 cm/hr)
Gradient	As indicated.
Temperature	30°C
Detection	280 nm
Injection volume	5 µl
Sample:	IgG, IgA, IgM

Often times the presence of high levels of IgG can either mask or falsely identify the presence of IgM or IgA which could otherwise be an early indication of the onset of a disease.
Since early diagnosis is essential in treatments, the use of a fast prefractionation method such as the one described in the present application note can be pivotal in eliminating the shortcomings of electrophoresis procedures.
The present method can be adapted to SPE micro-cartridges and hyphenated with a 96-well plate. Similarly, the well-plate system could be used as a front-end purification/concentration step for LC-MS-MS to improve the sensitivity of MS detection in a shorter time.