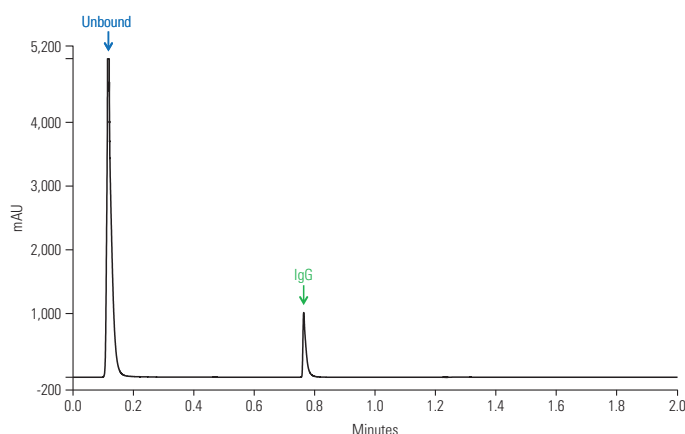


Affinity Titer Determination of mAbs



Early in the development of recombinant mAbs, a large number of harvest cell culture (HCC) samples must be screened for IgG titer. The high degree of specificity offered by affinity chromatography employing Protein A provides a powerful alternative to immunoaffinity methods such as ELISA.

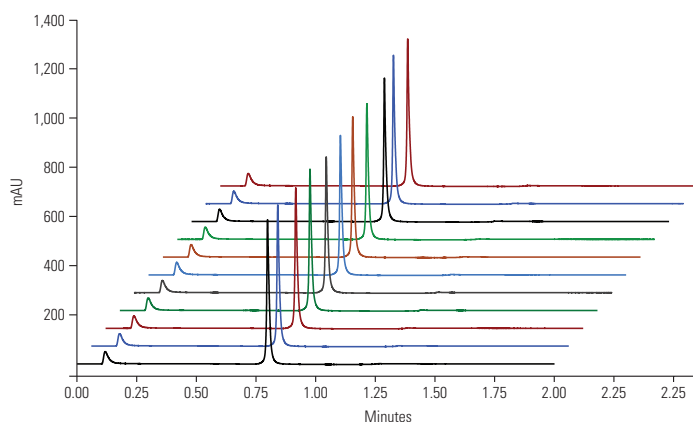
Protein A is often used due to its strong affinity to bind a wide range of antibodies. By linking Protein A to a chromatographic media, an affinity column can be created to determine the mAb concentration in HCC.



Column:	MABPac Protein A , 12 μ m 4.0 \times 35 mm
Mobile Phase A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Mobile Phase B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Flow Rate:	2 mL/min
Inj. Volume:	10 μ L
Temp:	25 $^{\circ}$ C
Detection:	280 nm
Sample:	Harvest cell culture (HCC)

The challenge facing the analytical laboratories in the pharmaceutical industry is to develop a high-throughput and robust titer assay. The recently developed novel column specifically for this application, Thermo Scientific™ MABPac™ Protein A has been engineered from a novel polymeric resin with a hydrophilic surface, covered with a covalently bound recombinant Protein A.

The hydrophilic nature of the backbone minimizes non-specific binding, and therefore enables accurate quantification of the mAb titer. The MABPac Protein A column format allows rapid automation of loading, binding, elution and collection using biocompatible systems from Thermo Fisher Scientific.



Column:	MABPac Protein A , 12 μ m 4.0 \times 35 mm
Mobile Phase A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Mobile Phase B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Flow Rate:	2 mL/min
Inj. Volume:	20 μ L
Temp:	25 $^{\circ}$ C
Detection:	280 nm
Sample:	Rabbit IgG, 1 mg/mL

	t_r (min)	Area (mAU*min)	PWHP (min)
Average	0.80	7.76	0.01
% RSD	1.02	2.20	0.00