

## Noise level comparison of gel filtration columns with inline light-scattering detection on ÄKTA design system

### Introduction

Light-scattering (LS) detection is used to determine the molecular weight and hydrodynamic radius of biomolecules after separation by gel filtration. Accurate results require a stable baseline during analysis. As LS detectors are sensitive to particles in solution, filtering sample and buffers before use is essential. Particles may also be generated by the packed column itself. Experience shows that all columns shed particles to some extent, and that shedding may be most noticeable in columns used for the first time.

We have investigated noise levels obtained when running a new Tricorn™ Superdex™ 75 10/300 GL gel filtration column (17-5174-01) on ÄKTAexplorer™ 10 chromatography system connected to an inline LS detector. We also compared a Superdex HR 10/30 column (now replaced by the new Tricorn column) and an equivalent TSK-GEL™ column (G2000SWXL, Tosoh Bioscience). See Table 1 for details.

### Experimental

ÄKTAexplorer 10 system included UNICORN™ 5.01 control software, a 1.0 µm inline filter, an inline degasser, and a Wyatt miniDawn™ Tristar LS detector with angles 41.5°, 90° and 138.5° for photodiodes 1, 2 and 3 respectively (Wyatt Technology). Buffer was 20 mM sodium phosphate, 0.28 M NaCl, 6 mM KCl, pH 7.4. The sample comprised proteins from the Gel Filtration LMW Calibration Kit (17-0442-01).

Each new column was fitted inline and equilibrated overnight with buffer to shed particles. Each was then switched to by-pass mode to check the baseline noise level without it (i.e. just the system effect at low pressure). Finally, each was reconnected inline, equilibrated with two column volumes of buffer, injected with the sample mixture and eluted. Baselines were measured at the point of minimum noise after the overnight run, during by-pass mode and after sample peak elution.

According to the LS detector manufacturer (Wyatt), an acceptable average baseline noise is ~15 mV at angles greater than 90° and ~10 mV at angles less than 90°.

### Results

All columns displayed low noise levels (< 10 mV at 90°) after overnight washing. Baselines were higher (10–30 mV at 90°) when the LS detector was run without the column. This is mainly an effect of the lower pressure in systems with columns disconnected. After sample peak elution, the following average noise levels were measured at 90°: Tricorn Superdex 75 10/300 GL, 8 mV (Fig. 1A); Superdex 75 HR 10/30, 15 mV (data not shown); TSK G2000SWXL, 30 mV (Fig. 1B).

### Conclusions

Particle shedding from new columns can easily be minimized by overnight running in equilibration buffer. Tricorn Superdex 75 10/300 GL showed low baseline noise levels and was well within Wyatt's acceptance criteria for 90°. Tricorn columns will thus give good results on ÄKTAexplorer 10 system fitted with an inline Wyatt LC detector. In this study, the TSK column showed noise levels above the criteria of acceptance for 90°.

Table 1. Gel filtration columns used to investigate baseline noise levels in the LS detector

Column	Column (id x bed size (µm))	Average particle Size
Tricorn Superdex 75 10/300 GL*	10 x 300	13
Superdex 75 HR 10/30*	10 x 300	13
TSK-GEL G2000SWXL	7.8 x 300	5

\* Packed with the same batch of medium.

For additional information on Tricorn Superdex columns, download data file 18-1163-79 at [www.amershambiosciences.com/promo\\_dm206](http://www.amershambiosciences.com/promo_dm206)

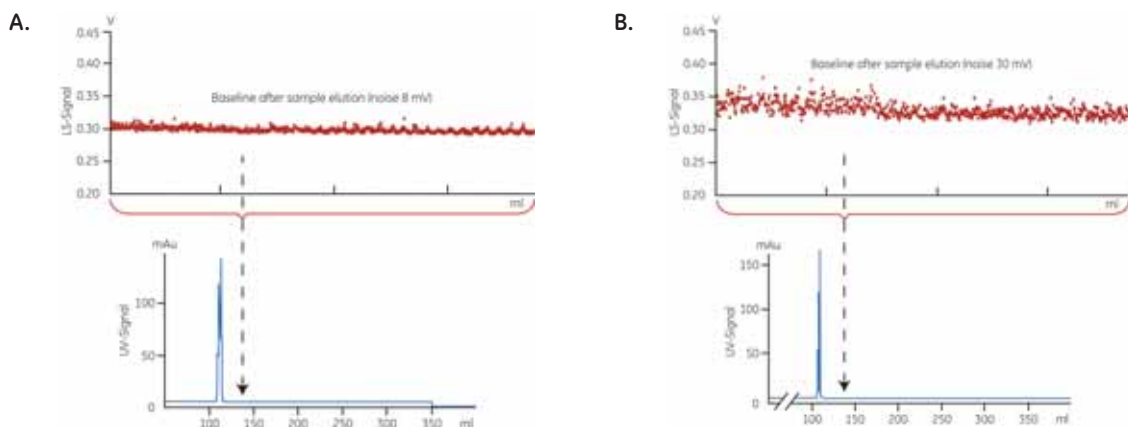


Fig 1. UV trace of the gel filtration separation and the magnified LS detector signal (90°) with the column inline after sample elution. (A) Tricorn Superdex 75 10/300 GL. (B) TSK-GEL G2000SWXL. Average noise level is given in mV.