TECHNOTE 102

Purification of Nanoparticles by Size Exclusion Chromatography (SEC)

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Introduction

Micromod's nanoparticles are most frequently supplied in aqueous suspension without any surfactants. Our protein-coated particles come in PBS buffer and sodium azide as antimicrobial agent.
All our magnetic, fluorescent and white nanoparticles with diameters < 130 nm can easily be purified from small molecules or salts with molecular weights < 1000 Da or transferred into special buffers by size exclusion chromatography (SEC). This purification method allows a quick removal of excess of reagents after activation or functionalization of chemical groups on the particle surface. SEC is an adjuvant alternative to ultracentrifugation, that prevents sonication to resuspend the pellet, especially if sensitive biomolecules are present in the system.

Handling Instructions

Desalting columns, that are based on gel filtration technique are available from different commercial suppliers. We recommend the use of desalting columns with an exclusion limit of 5.000 Da, e.g. PD-10 desalting columns for the purification of sample volumes up to 2.5 ml, PD Miditrap® G-25 columns for volumes up to 1 ml or PD Minitrap® G-25 columns for volumes up to 0.5 ml (all from GE Healthcare).
The columns can be used according to manufactures guidelines. Thereby the gravity protocol is favoured over the spin protocol. The equilibration of the desalting column with your buffer of choice is essential to remove any stabilizers, that are used in column packing. The particle suspension has to be diluted to the sample volume, that is required for the special column type (e.g. 2.5 ml for PD-10 desalting columns). The solid concentration of the particle suspension should not exceed 35 mg/ml to prevent any overloading of the column. The particle suspension should enter the packed bed of the column completely. The flow-through is discarded. Then the purified particles are eluted with the elution buffer into a test tube, that is placed under the column (e.g. 3.5 ml elution buffer for PD-10 desalting columns). By using the gravity protocol the particle suspension becomes diluted. The recovery of the applied particles is usually in the range of 80-95%.