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TECHNOTE 101

Separation of Magnetic Particles

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Introduction

Micromod's magnetic nano- and microparticles are most frequently supplied in aqueous suspension without any surfactants. Our protein-coated particles are dispersed in PBS buffer and sodium azide as antimicrobial agent. Magnetic particle separation with permanent magnets is a rapid method, if the magnetic particles have to be transferred into another medium or simply washed before the application.
Our magnetic particles with diameters > 130 nm can easily be separated with permanent magnets. All smaller nanoparticles of the product lines nanomag®-D-spio, nanomag®-CLD-spio, perimag® and synomag®-D as well as BNF particles with a diameter of 80 nm cannot be separated with conventional permanent magnets. Therefore the use of magnetic columns with high gradient magnetic fields is recommended. Alternatively these particles can be transferred into other media by size exclusion chromatography (see Technote 102) or dialysis (see Technote 103).
The 100 nm BNF particles are the smallest nanoparticles in micromod's assortment that can be separated with strong permanent magnets in a reasonable time.
Centrifugation of BNF, perimag® and nanomag® particles should be strictly prevented, because the structure of these composite particles would change at centrifugation.

Devices for magnetic separation

1. Separation of magnetic particles with diameters > 300 nm (micromer®-M, PLA–M particles, sicastar®-M, sicastar®-M-CT, and corresponding fluorescent magnetic particles)

The following table provides a selection of commercially available magnetic separators for our magnetic particles > 300 nm. This selection is not complete. Magnetic separators with similar properties can be used as well.

Magnet	Supplier	Description
Sepmag®	Sepmag Tecnologies	- easy scale-up to 5 L and more - separation in closed systems with process control
DynaMag®	Life Technologies® / Dynal	- different suspension volumes from 10 µl to 50 ml - separation in closed sterile blood bags
BioMag®	Bangs Laboratories, Inc.	- different suspension volumes from 1,5 ml to 50 ml - separation in 96 well plates
LifeSep®	Dexter Magnetic Technologies	- easy scale-up from 1.5 ml to 5 L and more - separation in 96 well plates - custom-specific design of magnets
SpeedSep®	Dexter Magnetic Technologies	- bulk magnetic separation for volumes up to 5L

2. Magnetic nanoparticles with diameters of 130 – 500 nm and 100 nm BNF-Starch particles

The magnetic separation of our 130 - 250 nm nanoparticles and of our 100 nm BNF-Starch particles requires stronger magnetic separators to achieve sufficient magnetic separation times. Such stronger magnetic separators are available for example at Dexter Magnetic Technologies. Dexter provides the LIFESEP® SX series of biomagnetic separators for single tubes/vessels for different volumes of magnetic particle suspensions from 1.5 ml to 5 L.
The LIFESEP® 1.5S for volumes of up to 1.5 ml and the LIFESEP® 15SX for separation of magnetic particles from volumes up to 15 ml were tested for the separation of nanomag®-D particles with diameters of 130 - 250 nm and of 100 nm BNF particles. The separation time of nanomag®-D particles at the LIFESEP® 15SX increases with decreasing particle diameter from 1 min for 500 nm nanomag®-D to 10 min for 250 nm nanomag®-D and about 20 min for 130 nm nanomag®-D. Within these separation times 100 % of the 500 nm nanomag®-D and more than 90 % of the 130 nm and 250 nm nanomag®-D particles are separated.

The separation of plain and functionalized 80 nm and 100 nm BNF particles was studied with the LIFESEP® 15SX. Therefore each 12 ml of BNF particle suspension with an iron concentration of 6 mg/ml were filled into a 15 ml tube and placed in the magnet. The iron concentration of the supernatant was measured over a period of 24 h as basis for the calculated of the amount of separated nanoparticles over time. The separation behaviour of BNF-Starch and BNF-Dextran particles is comparable.

Time [h]	Percentage of separated BNF particles [%]			
	Particle diameter: 100 nm		Particle diameter: 80 nm	
	Particle Surface: plain	Particle Surface: NH_2	Particle Surface: plain	Particle Surface: NH_2
0	0	0	0	0
0,5	74	99	57	42
1	93	100	71	66
1,5	97		79	79
2	98		86	84
3	100		91	93
4			96	96
5			97	96
6			98	97
7			98	98
24			100	100

The corresponding experiment was performed to study the separation of BNF particles at the LIFESEP® 1.5S. With this smaller magnetic device the following times to separate more than 90 % of BNF particles were determined:

Particle Diameter	Surface	Separation time
100 nm	plain	6 h
100 nm	NH_2	1 h
80 nm	Plain	> 8 h
80 nm	NH_2	> 8 h

The LIFESEP® 15SX can be recommended for the separation of 100 nm BNF particles in a reasonable time: More than 90 % of plain and 100 % of functionalized 100 nm BNF particles are separated at the magnet within 1 hour.
The LIFESEP® 1.5S can only be recommended for the separation of functionalized 100 nm BNF particles with a separation time of 1 h.

3. Superparamagnetic nanoparticles (nanomag®-D-spio, nanomag®-CLD-spio, perimag®, synomag®-D) and 80 nm BNF-Starch particles

For magnetic separation of nanoparticles of the product lines nanomag®-D-spio, nanomag®-CLD-spio, perimag® and synomag®-D as well as of BNF particles with a diameter of 80 nm the MACS® Magnetic Cell Separation device (Miltenyi Biotec GmbH) can be used. The MidiMACS® or QuadroMACS® sorting device with LS or LD columns is recommended.

The recovery rate of different types of magnetic nanoparticles was determined for LD columns in the QuadroMACS® device. Therefore the LD columns were pre-washed with 5 ml water or buffer. Then the column was placed in the QuadroMACS®, filled with 5 ml particle suspension and eluted. 5 ml of water or buffer were filled in the column and eluted. Finally the column was removed from the magnet and eluted with 5 ml water or buffer. The particle recovery was measured by determination of the iron concentration of the particle suspension before and after magnetic separation:

Particle type	Diameter	Surface	Product code	Recovery Rate
nanomag®-D-spio	20 nm	plain	79-00-201	≥ 70 %
nanomag®-D-spio	50 nm	plain	79-00-501	≥ 60 %
nanomag®-D-spio	100 nm	plain	79-00-102	≥ 80 %
nanomag®-D-spio	20 nm	COOH	79-02-201	≥ 60 %
nanomag®-D-spio	50 nm	COOH	79-02-501	≥ 60 %
nanomag®-D-spio	100 nm	COOH	79-02-102	≥ 60 %
nanomag®-CLD-spio	20 nm	NH_2	77-01-201	≥ 70 %
nanomag®-CLD-spio	100 nm	NH_2	77-01-102	≥ 80 %
perimag®	130 nm	plain	102-00-132	≥ 90 %
perimag®	130 nm	NH_2	102-01-132	≥ 90 %
synomag®-D	50 nm	plain	104-00-501	≥ 90 %
synomag®-D	50 nm	NH_2	104-01-501	≥ 90 %
BNF-Starch	80 nm	plain	10-00-801	≥ 90 %
BNF-Starch	80 nm	NH_2	10-01-801	≥ 90 %
BNF-Dextran	80 nm	plain	84-00-801	≥ 90 %
BNF-Dextran	80 nm	NH_2	84-01-801	≥ 90 %

Perimag®, synomag®-D and 80 nm BNF particles can be separated with LD columns in the QuadroMACS® device with a recovery rate of ≥ 90 %. The recovery rate of nanomag®-D-spio or nanomag®-CLD-spio is dependent on the particle diameter and surface modification and lies in the range of 60–80 %.