



**nanomag® -D-spio, Ø 20 nm, 50 nm or 100 nm**  
**Covalent binding of proteins/ antibodies by cyanogene bromide activation**  
**(CNBr-Method)**

Particle type: nanomag® -D-spio  
**Product-code: 79-00-201 / 79-00-501 / 79-00-102**  
Particle diameter: 20 nm / 50 nm /100 nm  
Particle surface: plain

Material:

- 10 ml nanomag® -D-spio suspension (surface: plain, c = 10 mg/ml)
- 0.2 M Na<sub>2</sub>CO<sub>3</sub>
- 0.1 M NaHCO<sub>3</sub>
- 5 M CNBr-solution in acetonitrile
- PBS buffer, pH=7.4
- 25 mM glycine in PBS buffer

Device:

- high gradient magnetic field (HGMF)

Procedure:

- separate the nanomag® -D-spio particles (10 ml, 10 mg/ml) in a high gradient magnetic field device (HGMF) and transfer it into 0.2 M Na<sub>2</sub>CO<sub>3</sub>-buffer
- measure the particle concentration by gravimetry (take care on the buffer concentration of 21.2 mg/ml), calculate the mass of particles m by multiplying volume and concentration
- calculate the mass of protein (antibody) for conjugation:  $m(\text{protein}) [\mu\text{g}] = m [\text{mg}] \times 8 \mu\text{g}/\text{mg}$
- dissolve the calculated amount of protein or antibody in 1 ml 0.1 M NaHCO<sub>3</sub>-buffer
- calculate the volume of 5 M CNBr solution:  $V(\text{CNBr}) [\mu\text{l}] = m [\text{mg}] \times 0.36 \mu\text{l}/\text{mg}$
- add the calculated volume of 5 M CNBr solution to the particle suspension
- shake for 10 min at room temperature
- wash the particles at HGMF with 0.1 M NaHCO<sub>3</sub>
- elute the particles with 0.1 M NaHCO<sub>3</sub>
- add the protein or antibody solution to the suspension
- shake the suspension over night by room temperature
- add 1 ml of 25 mM glycine in PBS buffer
- shake for 30 min at Room temperature
- wash the particles at HGMF with PBS buffer

The protein binding capacity of nanomag® -D-spio particles is about 2.5-3 µg/mg for albumin (BSA), protein A, avidin and streptavidin.