TECHNOTE 301

Determination of the amount of covalently bound proteins on the surface of magnetic nanoparticles

micromod Partikeltechnologie GmbH
Schillingallee 68
D-18057 Rostock
Germany

Telephone: + 49 381 / 79 99 70 00
Fax: + 49 381 / 79 99 70 70
E-mail address: info@micromod.de

Introduction

Magnetic nanoparticles strongly influence the background signal of protein determination by bicinchoninic acid assays. This background signal should be reduced by the following method. For the successful application exactly the same iron concentration of the reference and protein coated particles are essential. This assay was successfully used for the determination of the antibody concentration on conjugated nanomag®-CLD-spio [1] and BNF particles [2, 3].

Protocol

Material:

- 200 µl suspension of protein coated magnetic nanoparticles, c(Fe) = 0.5 mg/ml*
- 500 µl suspension of reference particles (before coating), c(Fe) = 0.5 mg/ml*
- PBS buffer (pH=7.4), Sigma, P3813
- Protein stock solution
- Micro BCA Reagent A (MA), Pierce, 23231
- Micro BCA Reagent B (MB), Pierce, 23232
- Micro BCA Reagent C (MC), Pierce, 23234
- 96 well microplate + microplate reader (570 nm)

*At lower protein loadings iron concentrations up to 1 mg/ml can be used.

Procedure:
Calibration curve:

- Prepare 1 ml protein-50 standard solution, c = 50 µg/ml, by dilution of the protein stock solution with PBS buffer
- Fill wells B – F with each 150 µl PBS-buffer,
- Add 150 µl protein-50 solution into wells A and B,
- Transfer 150 µl from well B into well C and mix well,
- Continue this transfer from well C to D and from well D to E,
- Discard 150 µl from well E. Well F contains pure buffer,
- Fill each 75 µl of the reference particle suspension into the wells A to F of the neighbor row,
- Add 75 µl of the protein dilutions into the wells with the reference particle samples (A to A, B to B etc.).
- The protein concentration in the single wells of the calibration curve is as follows:

Well	c (Protein) [µg/ml]	Well	c (Protein) [µg/ml]
A	25	D	3,125
B	12,5	E	1,5625
C	6,25	F	0

Sample measurement:
Fill 75 µl of the suspension of protein coated magnetic nanoparticles each into two other wells, and add each 75 µl PBS buffer to these wells.

Preparation of BCA development solution:
For each well 150 µl of BCA solution are necessary.
The development of the calibration curve requires 1 ml BCA solution.

V (BCA solution) [µl] = number of sample wells x 150 µl + 1000 µl
V (solution C) [µl] = V (BCA solution) [µl] / 50
V (solution B) [µl] = V (solution C) [µl] x 24
V (solution A) [µl] = V (solution C) [µl] x 25

Add 150 µl of freshly prepared BCA solution to each filled well and incubate the microplate for 2 hours at 37°C. Measure the absorbance at 570 nm in a microplate reader.
Calculate the protein concentration per mg Fe of the analyte samples by comparison with the calibration curve.

References:

1. Al Faraj, A., et al., MR imaging and targeting of a specific alveolar macrophage subpopulation in LPS-induced COPD animal model using antibody-conjugated magnetic nanoparticles. *Int. J. Nanomed.*, **2014**. **9**: p. 1491-1503.
2. Al Faraj, A., et al., Specific targeting and noninvasive magnetic resonance imaging of an asthma biomarker in the lung using polyethylene glycol functionalized magnetic nanocarriers. *Contrast media & molecular imaging*, **2015**.
3. Baiu, D.C., et al., High specificity targeting and detection of human neuroblastoma using multifunctional anti-GD2 iron-oxide nanoparticles. *Nanomedicine*, **2015**, **10**(19): p. 2973-2988.