



## Small molecules as anchor coated to magnetic beads

### Customized heme coated magnetic beads for pull out experiments

**Small molecules covalently coated to magnetic beads recently became very popular in several life science disciplines incl.:**

- chemical proteomics
- biomarker discovery
- signal transduction pathway analysis
- fishing for target proteins for target ID and/or small or large scale purification

In this application note we will outline the results from one of our clients, Prof. James Allen, from the Oxford University, Department of Biochemistry. One of his main interest are heme proteins, since heme (Fe-protoporphyrin IX) is a critical cofactor, found in Archaea, Bacteria and Eukarya. Hemoproteins play numerous roles, including oxygen transport (e.g., hemoglobin, myoglobin), detoxification (e.g., cytochrome P450), gas sensing (e.g., CooA), electron transport (e.g., cytochrome c), catalysis (e.g., cytochrome oxidase, cytochrome cd1 nitrite reductase) and in protein assembly (e.g., CcmE).

Prof. James Allen was interested in a generic pull out system to analyze protein-heme interactions and heme trafficking systems within a living cell. Therefore MagnaMedics has been asked to synthesize specifically for his research heme coated magnetic beads with very distinct specifications:

- the particles should contain a high magnetic content for fast separation in a magnetic field.
- a distinct heme concentration should be covalently attached to magnetic beads, low enough to avoid unspecific heme/heme interactions, and high enough to get a high sensitivity in the assay.
- heme should be coated by a long spacer for best sterical access of the target protein towards the functional heme group.

MagnaMedics provided James Allen with the following customized MagSi beads:

- $5 \times 10^9$  MagSi beads/ml bead solution
- bead size: CI 90% 0.7 – 1.4  $\mu\text{m}$
- apr.  $6 \times 10^5$  heme molecules conjugated per bead.

A typical analysis was conducted as follows: 50  $\mu\text{l}$  heme-conjugated beads (from a suspension of  $5 \times 10^9$  beads/ml) were collected using the magnet then washed in PBS buffer. The beads were re-suspended in 200  $\mu\text{l}$  of the sample to be analysed (giving an effective heme concentration of 1.25  $\mu\text{M}$ ) and allowed to incubate for at least 15 min (but see below) at 20°C. The resulting bead-heme-bound protein complex was then collected using the magnet and washed four times using 200  $\mu\text{l}$  PBS buffer for each wash. The beads were re-suspended in 50  $\mu\text{l}$  PBS and transferred to a fresh tube, then collected using the magnet. Where reducing conditions were required, a few grains of fresh disodium dithionite were added to the



sample for analysis and to the first two washes. Elution of bound proteins was either performed with SDS-PAGE loading buffer or addition of imidazol. The bound, washed and eluted protein have been analysed by SDS-PAGE.

As result a stringent, sensitive assay for analysis heme/protein complexes could be established (Fig.1)

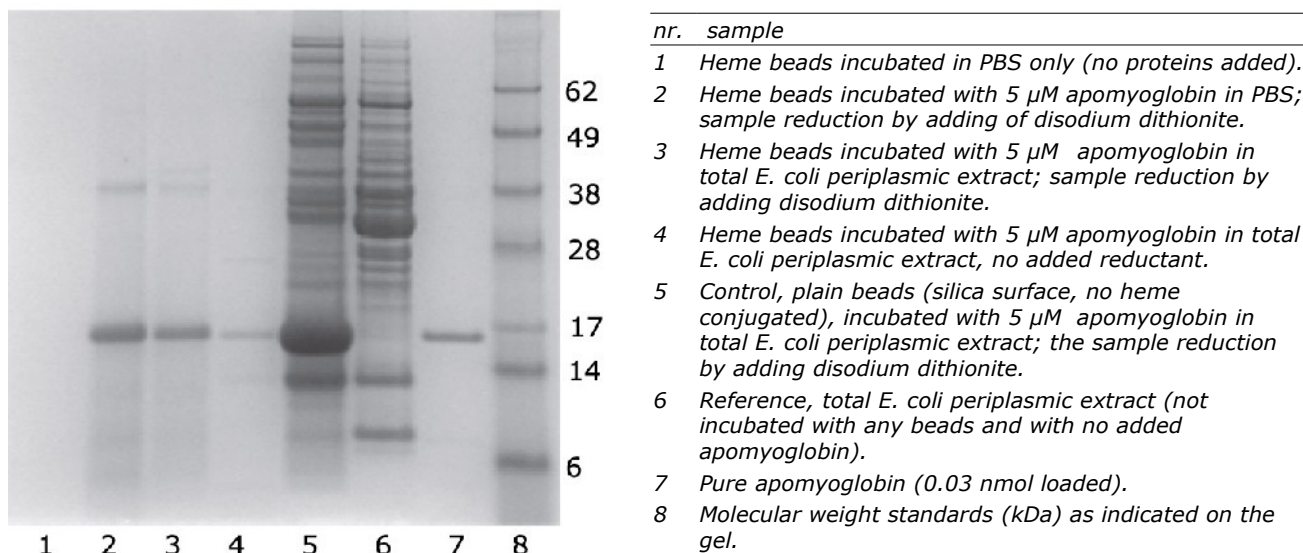


Fig. 1.: SDS-PAGE analysis of proteins isolated using heme conjugated magnetic beads. Samples were incubated with the beads for 20 min. The beads were then washed four times with buffer using magnetic separations, re-suspended in 20  $\mu$ l buffer and eluted by boiling for 15 min. in SDS-PAGE sample buffer. The gel was stained with Simply Blue SafeStain.

For further details on the assay please follow: „Heme conjugated magnetic beads to isolate heme-binding proteins from complex mixtures“, Rhian Jones, James W.A. Allen; Protein Expression and Purification, 2010, in press.

Please note: MagnaMedics is offering customized magnetic and non-magnetic bead bead development, incl. coatings of individual small molecules.

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