

Magnetic particles as powerful biomarker discovery tool

In this article MagnaMedics Diagnostics B.V. will outline the use of MagSi products for biomarker discovery with emphasis on latest proteomics approaches known today.

- ***chemical proteomics research***
- ***nuclear receptor research***
- ***signal transduction pathway analysis research***

Biomarker discovery in general

Protein biomarkers remain appealing to drug developers because of the promise of early elimination of ineffective or toxic compounds during medicine development. Also the lead time during target validation, and creation of cellular assays for drug discovery can be improved. However, the difficulty and costs of characterizing and validating new biomarkers has held back rapid development in this field. Now, with the MagSi biomagnetic particle product line, beads are providing new ways to identify biomarkers faster, more efficiently, and cheaper. MagnaMedics delivers products with a discrete and controlled particle size distribution, with unique surface chemistry QC-AC protocols. MagnaMedics Diagnostics enables scientist to obtain results without having to worry about batch to batch differences between MagSi products used. MagnaMedics Diagnostics enables investigators to address the critical parameters for particles to be used in such a fashion that research requirements are addressed without limits.



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Table 1: General overview on MagSi products suitable for biomarker discovery research

Product	Description	Used for	Remarks
MagSi-protein A MagSi-protein G	MagSi magnetic silica particles with high quality, recombinant Protein A or G covalently bound to the particle surface	Antibody purification, antibody → protein complex isolation and purification	Available in standard volumes of 1 and 5 ml, 10 mg / ml, 600 nm or 1 µm standard sizes
MagSi-STA	MagSi magnetic silica particles with a mono layer of high quality streptavidin covalently attached to the bead surface	Coupling of biotinylated ligands, antibodies and other biomolecules for e.g., binding assays etc.	Available in standard volumes of 2 and 10 ml, 10 mg / ml, 600 nm or 1 µm standard sizes
MagSi-proteomics	MagSi magnetic silica particles with modified C4, C8 or C18 surface.	Peptide/protein profiling, desalination for MALDI on basis of hydrophobic - hydrophilic properties of target material	Available in standard volumes of 2 and 10 ml, 10 mg / ml, 1,2 µm standard size
MagSi-COOH	MagSi magnetic silica particles coated with a carboxyl group containing polymer, covalently attached to bead surface	Covalent coupling of (bio-) compounds.	Available in standard volumes of 2 and 10 ml, 10 mg / ml, 600 nm or 1 µm standard sizes
MagSi-WCX	MagSi magnetic silica particles with weak cation exchange surface (WCX)	Peptide/protein profiling, desalination for MALDI on basis of acidic - basic properties of target material (pI values)	Available in standard volumes of 2 and 10 ml, 10 mg / ml, 1,2 µm standard size
MagSi-WAX	MagSi magnetic silica particles with weak anion exchange surface (WAX)	Peptide/protein profiling, desalination for MALDI on basis of acidic - basic properties of target material (pI values)	Available in standard volumes of 2 and 10 ml, 10 mg / ml, 1,2 µm standard size
MagCustom	Tailor made particles	Specific assays and research	Heme, GOX and others

Chemical proteomics

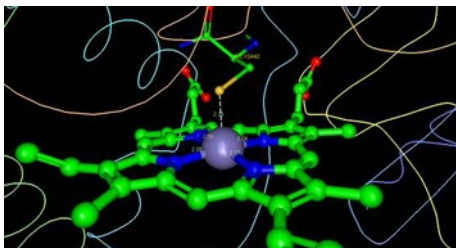


Figure 2: Interaction of the protein ligand thiolate and Fe

The medical and pharmaceutical communities are facing a dire need for new drugable targets, while, paradoxically, the targets of some drugs that are in clinical use or development remain elusive. Many compounds have been found to be more promiscuous than originally anticipated, which can potentially lead to side effects, but which may also open up additional medical uses. As we move toward systems biology and personalized medicine, it will become increasingly necessary to comprehensively determine small molecule– target interaction

profiles and to map these on signalling and metabolic pathways. Chemical proteomics is a powerful mass spectrometry based affinity chromatography approach for identifying proteome-wide small molecule–protein interactions. Using magnetic particles reduces time and costs in the screening processes during these studies. As magnetic beads are highly automatable, setting up possibilities for HT screening are potentially achievable. Specific reference in which MagnaMedics' products were used in chemical proteomics research are addressed [here](#).

Table 2: General overview on MagSi products suitable for chemical proteomics research use

Cat. No.	Product	Use	Remarks
MD16004	MagSi-COOH	Covalent coupling of small organic molecules	Usually in MagCustom project
MD80001	MagCustom phase I study	Specific ligand binding project to identify proteome-wide small molecule–protein interactions	Examples: Heme, Lectin etc

Nuclear receptor research

In the field of molecular biology, nuclear receptors are a class of proteins found within cells that are responsible for sensing steroid and thyroid hormones and certain other molecules. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism. Many of these regulated genes are associated with various diseases, which explains why the molecular targets of approximately 13% of U.S. FDA approved drugs are nuclear receptors.

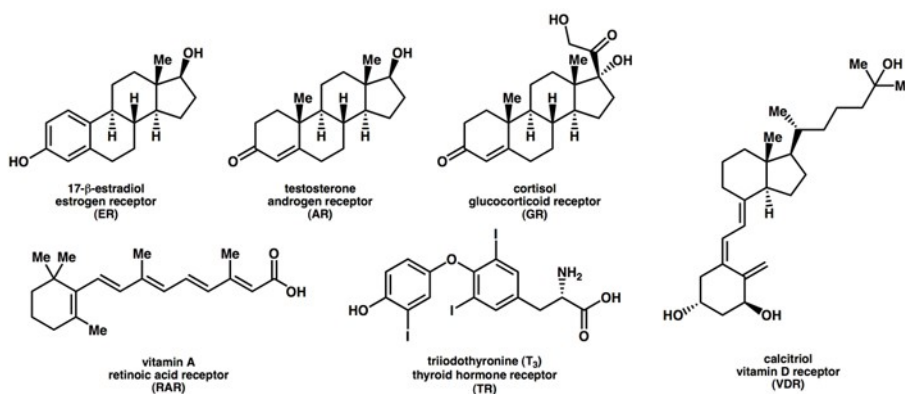
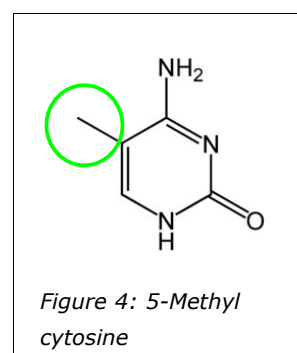


Figure 3: Structures of selected endogenous nuclear receptor ligands and the name of the receptor bound

Identification of the receptors so far revealed in humans, mice and rats at least 48, 49 and 47 receptors respectively. As receptors regulate the expression of specific proteins release in the cell ER, it is a powerful source of interest for drug target discovery. Through coupling of biotinylated DNA sequences to MagSi-STA particles, researchers can swiftly screen the abundance of proteins binding these sequences in such a fashion that it can be done in a HT set-up. The use of 600 nm beads potentially limits the compromising effects of sedimentation of beads during the binding experiment. As the binding affinity of the proteins for specific DNA sequences is not (always) specified, shaking of the beads - lysate mixture during the binding assay, may influence the results negatively.

Other uses of magnetic beads in nuclear receptor studies

5-Methyl cytosine is a methylated form of DNA base cytosine. When cytosine is methylated, the DNA maintains the same sequence, but genes can be turned on and off (a mechanism called epigenetics). DNA methyl transferase enzymes are known to modify basic cytosine into this form. The biological significance of cytosine methylation is as yet incompletely understood. However, substantial and growing evidence strongly suggests that perturbation of methylation patterns, resulting from the infidelity of DNA cytosine methyl transferase, is an important component in the development of human cancer.



MagnaMedics Diagnostics has coupled specific monoclonal antibodies against 5-Methyl cytosine generating a powerful tool for researchers to screen for and select not yet discovered methylated base patterns potentially related to cancer development. It generates a powerful tool for sequence analysis were methylation may play an important role in gene regulation and development of cancer and other diseases.

Table 3: General overview on MagSi products suitable for nuclear receptor research use

Cat. No.	Product	Use	Remarks
MD16001 MD01001	MagSi-STA	Covalent binding of biotinylated DNA for screening assays on nuclear receptors	MagSi-STA 600 eliminates sedimentation of beads during binding of DNA->Protein
MD01014 MD01015 MD01009	MagSi-proteomics	Peptide/protein profiling and purification focussing on hydrophobic hydrophilic properties of molecules involved	Range of products comprising MagSi-proteomics C4, C8 and C18
MD01023	MagSi-WCX	Peptide/protein profiling, desalination for MALDI on basis of acidic <-> basic properties of target material (pI values)	Advise is to test both types in parallel to select best fitting type
MD01025	MagSi-WAX	Peptide/protein profiling, desalination for MALDI on basis of acidic <-> basic properties of target material (pI values)	Advise is to test both types in parallel to select best fitting type
MD80001	MagCustom	Specific ligand binding	Examples: Directly coupled antibodies, hormones, etc.

Signal transduction pathway analysis research

Cell signalling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions.

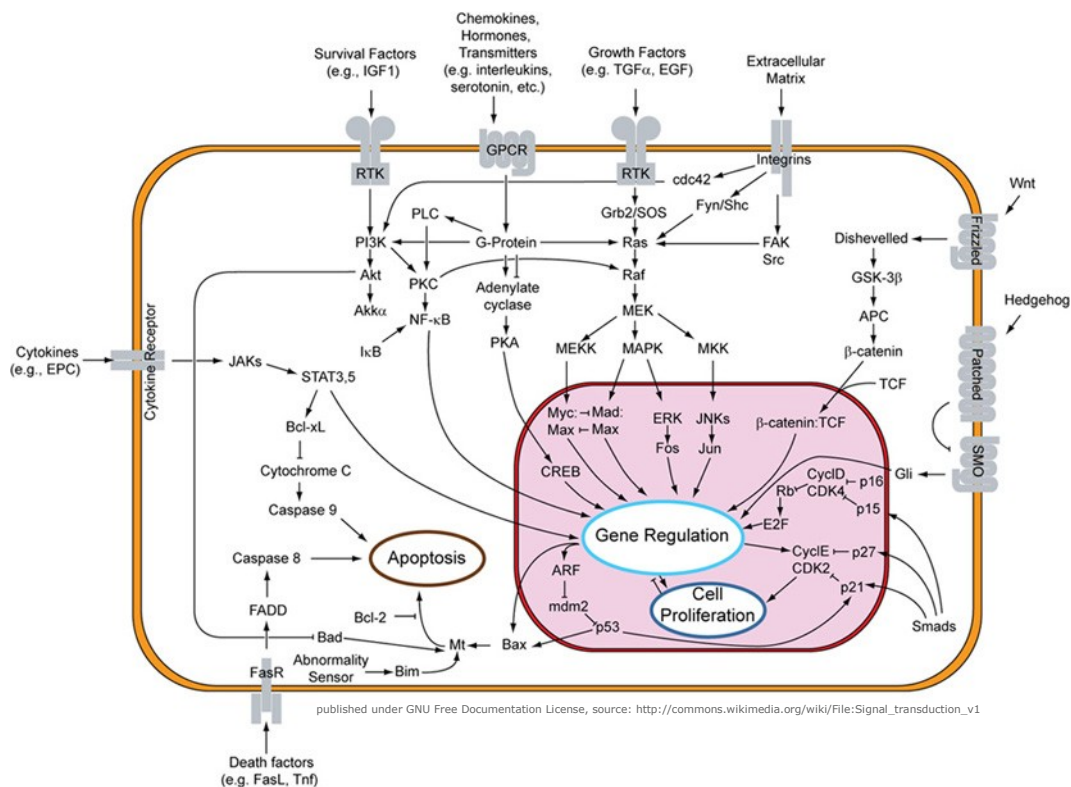


Figure 5: Overview of signal transduction pathways

Molecules that activate (or, sometimes, inhibit) protein receptors identified called hormones, neurotransmitters, cytokines or growth factors, together they are classified as receptor ligands. The details of ligand-receptor interactions are fundamental to cell signalling, therewith to drug target discovery.

MagnaMedics Diagnostics offers researchers possibilities too select specific agents and targets for coupling to magnetic beads, either directly (MagCustom), or indirectly via MagSi-protein A or G and MagSi-STA. MagnaMedics Diagnostics helps researchers to uncover critical pathways related to diseases via identification of relevant compounds involved in signal transduction pathways, and potential drugs influencing the cell response itself.

Use of directly coupled enzymes (in this case glucose oxidase) can be found in numerous [publications](#). As an example MagnaMedics has coupled this specific enzyme to MagSi beads, proving its capability in general to couple bio active compounds to the MagSi particles. The relative easy assay demonstrates the use in general of beads as platform for measurements of enzyme activity.

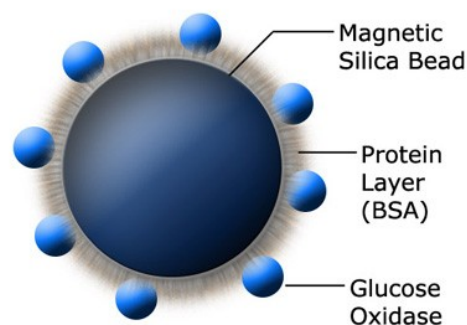


Figure 6: Glucose Oxidase coupled bead



In [literature](#), there is mention of several manners of direct enzyme coupling. If scientist would like enzymes to be coupled to MagSi-beads this can be arranged via a normal feasibility study as a first phase of a [MagCustom](#) project performed at Magnamedics Diagnostics. MagnaMedics Diagnostics will help interested parties to select the most appropriate method for immobilization of the enzyme in question. If you already have a good idea on what it is you need, fill out the question list published [here](#).

Table 4: General overview on MagSi products suitable for signal transduction pathway research use

Cat. No	Product	Use	Remarks
MD10011 MD10012	MagSi-protein A MagSi-protein G	Antibody, antibody-protein complex isolation	
MD16001 MD01001	MagSi-STA	Covalent binding of Ab's and biomolecules used in assays and IVD experiments	MMD has developed modifiable reproducible binding capacity ranges
MD01014 MD01015 MD01009	MagSi-proteomics	Peptide/protein profiling and purification focussing on hydrophobic - hydrophilic properties of molecules involved	Range of products comprising MagSi-proteomics C4, C8 and C18
MD16004	MagSi-COOH	For covalent coupling of (bio-)compounds	Usually used in MagCustom projects
MD01023	MagSi-WCX	Peptide/protein profiling, desalination for MALDI on basis of acidic - basic properties of target material (pI values)	Advise is to test MagSi-WAX in parallel
MD01025	MagSi-WAX	Peptide/protein profiling, desalination for MALDI on basis of acidic <-> basic properties of target material (pI values)	Advise is to test MagSi-WCX in parallel
MD80001	MagCustom	Specific ligand binding for target selection	Examples: Glucose Oxidase etc.

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