



The use of Magnosphere™ MS300/Tosyl paramagnetic beads in HCV and EBV assay formats.

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With chemistry, we can.

Abstract

Paramagnetic beads have been popular in the field of clinical diagnostics as a solid support because of their advantages in automation, ease of handling, and rapid separation. To obtain a sensitive assay using paramagnetic beads, it is very important to avoid non-specific binding from cell lysate proteins and serum. JSR Magnosphere™ beads consist of a core shell structure with three layers as can be seen in Figure 2. The JSR Magnosphere™ surface is coated with an even layer of JSR proprietary polymer. This gives the beads their characteristic low non-specific binding and ensures efficient shielding of the magnetite. The non specific binding of the JSR Magnosphere™ MS 300/Tosyl beads with a diameter of 3 µm have been tested in a HCV (Hepatitis-C Virus) and EBV (Epstein-Barr Virus) assay run on a fully automated, chemiluminescent immunoassay system. The particular HCV antigen is a recombinant protein, sensitive to heat stress if coated on beads. The HCV antigen was coated on both JSR and a competitor's beads. The performance of the beads was tested on 3 sample groups: HCV positive samples, a small population of HCV negative serum samples and a small number of HCV seroconversion samples. The coated beads were subject to heat stress by incubation at 37 °C for 3 and 6 days respectively. The reactivity of the coated beads after heat stress at 37 °C for 3 and 6 days was equal for both JSR and competitor beads; both of them seem quite stable until 3 days at 37 °C. It was seen that at the 6th day the response for both type of beads is lower. The reactivity of especially the seroconversion samples fall to intolerable values for the competitor beads, while the signal with the JSR beads is still easily recognized. The JSR Magnosphere™ MS300/Tosyl beads were also evaluated in an EBV assay on a number of non-EBV "cross-reacting" samples. It was observed that the JSR beads showed a much lower level of "cross-reacting" signal than the competitor's beads. In these particular assays the JSR Magnosphere™ MS300/Tosyl beads have proved to be interesting in terms of ability to stabilize a potentially temperature labile protein and in terms of low non-specific binding from "cross-reacting" samples.

JSR ParaMagnetic Beads

Synthesis of magnetic beads

JSR Magnosphere™ products series are superparamagnetic micro particles for IVD and research. The particles have a uniform size and core shell structure of Polymer and magnetite. Rotor blades were used for coating of Magnetite. Their surfaces are coated with an even layer of JSR proprietary polymer to give the beads their characteristic low non-specific binding and no surface exposure of the magnetite. Various surface functional groups such as Carboxyl, Tosyl, Streptavidin, Oligo-dT and beads sizes 3µm (300) and 1.5 µm (150) are available.

Magnosphere MS300/Carboxyl	Immunoprecipitation, ELISA
Magnosphere MS150/Carboxyl	
Magnosphere MS300/Streptavidin	PCR, DNA separation
Magnosphere MS150/Streptavidin	
Magnosphere MS300/Tosyl	ELISA, Cell Separation
Magnosphere MS150/Tosyl	
Magnosphere MS300/Oligo-dT	Poly-A separation
Magnosphere MS150/Oligo-dT	

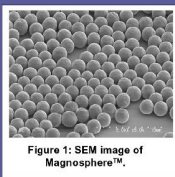


Figure 1: SEM image of Magnosphere™.

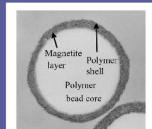


Figure 2: Cross section image of Magnosphere.

Performance of JSR Magnosphere™ MS/Tosyl beads in a HCV-assay.

Introduction and Test set-up:

The HCV antigen is a recombinant protein, sensitive to heat stress if coated on beads. In the described immunoassay, JSR Magnosphere MS300/Tosyl beads (diameter 3 µm) and competitor Tosyl paramagnetic beads were coated with the same HCV antigen at the same concentration and with the same coating protocol. Following serum samples were used:

- HCV positive samples (4) diluted 1:50 in serum/plasma HCV negative;
- A small population of HCV negative serum samples;
- A small number of HCV seroconversion points.

A stability trial of the coating was performed at 37 °C for 3 and 6 days, respectively. Afterwards the immunoassay measures were run at the same time with the same immunoassay instrument.

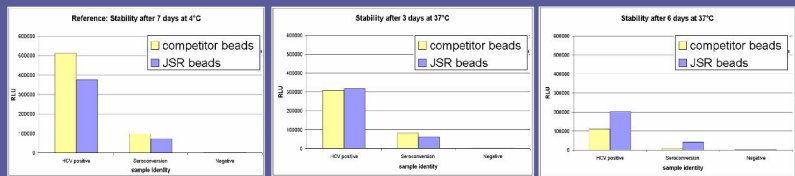


Figure 3: Stability of competitor and JSR Magnosphere MS/Tosyl beads coated with HCV antigen.

Results and Conclusion:

The reactivity of the coated JSR beads after heat stress at 37 °C for 3 and 6 days has the behaviour similar to the competitors beads; both of them seem quite stable until 3 days at 37 °C, although the negative populations appear to be better for JSR.

After heat stress at 37 °C for 7 days the reactivity of the seroconversion points fall to intolerable values of RLU's for the competitor beads, while with the JSR beads the reactivity of the same HCV seroconversion samples is reduced but not to the levels of the competitor beads. After 7 days of heat treatment at 37 °C it is still possible to recognize the seroconversion points with the JSR beads.

Performance of JSR Magnosphere™ MS/Tosyl beads in an EBV-assay for cross-reacting EBV samples.

JSR Magnosphere MS/Tosyl beads were tested in a number of crossreacting samples in an EBV (Epstein Barr Virus) assay. It was investigated in how far beads surface chemistry has an influence on the false positive signal. It is seen in Figure 4 that when non-antigen coated JSR Magnosphere MS 300/Tosyl beads were added to the cross-reacting (sample id: CR*) samples, the non-specific signal was much lower than with the competitor beads. However when the beads were coated with EBV antigen, the false positive sample of the cross-reacting samples was still unacceptable.

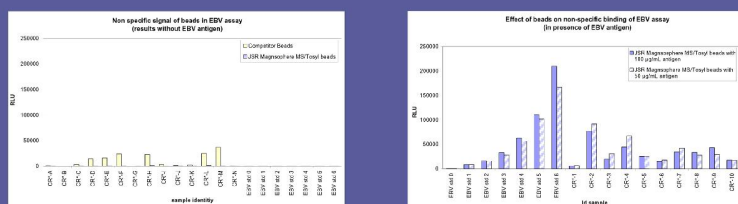


Figure 4: Effect of beads on non-specific binding reactions of crossreacting samples in an EBV assay.

Magnosphere™	Series	Surface Chemistry	Hydrophobicity	Particle size (µm)
MK Series	MK230/Carboxyl	Hydrophobic	2.1	
MB Series	MB100/Carboxyl	Hydrophobic	1.1	
	MB200/Carboxyl	Hydrophobic	2.0	
MS Series	MS300/Carboxyl	Hydrophilic	3.0*	
	MS300/Tosyl	Hydrophilic	3.0*	
	MS300/Streptavidin	Hydrophilic	3.0*	

* Also available with a particle size of 1.5µm

Conclusion:

JSR beads proved to be better performing in terms of Non Specific Binding, good positive reactivity and a notable improvement in thermal stability performance.