JSR Life Sciences

Rev. No.1

Magnosphere™ MS300/Carboxyl

PRODUCT DESCRIPTION

Magnosphere™ MS300/Carboxyl beads are magnetic microparticles designed for bioseparation. Their surfaces are covered with a JSR Life Sciences proprietary hydrophilic polymer, on which carboxy group is incorporated as an active group. The carboxy group makes it possible to immobilize ligands containing $-NH_2$ groups such as antibody or DNA through an amino coupling method. These chemistries enable ligands to keep their function high and achieve a high yield and low non-specific binding bioseparation.

These characters of the ***Magnosphere™ MS300/Carboxyl*** beads are ideal for a variety of applications such as enzyme immunoassay, immnoprecipitation, IP-western, and DNA hybridization,

Use of ***Magnosphere™ MS300/Low Carboxyl*** is also recommended for ultimately sensitive assay like a LC-MS assay of Immunoprecipitation.

FEATURES

- Uniform particle size
- Superparamagnetic
- Rapid magnetic responsiveness
- Low non-specific binding of proteins
- Surfactant free and oligomer free

EXAMPLE APPLICATIONS

Immunoassay, immunoprecipitation, Western blot, nucleic acid hybridization

SPECIFICATIONS

Package volume 4 mL
Solid content in slurry* 1 % (10 mg/mL, 6 x 10^8 beads/mL approx)
Dispersion media 0.01%ProClin950AI / H_2O
Bead diameter 3 μm
Bead magnetite content 20 % approx.
Surface charge density* 10 nmol/mg bead approx.
Shelf life Labeled on the bottle
*Surface charge density = amount of active functional group per 1 mg beads

STORAGE

Stored at 2-8 °C. Do not freeze the vial. Vortex the vial or pipette gently up and down to obtain a homogeneous dispersion before use.

RECOMMENDED PROTOCOLS

Three examples of chemical coupling protocol of antibody onto the ***Magnosphere™ MS300/Carboxyl*** beads are shown below. The optimum condition may depend on the toughness of the antibody used.

Reagent and equipment requirement

Binding Buffer:	0.1 M MES* buffer pH 5.0 (*MES: 2-(N-morpholino)ethanesulfonic acid)
Washing Buffer:	TBS-T (25 mM Tris-HCl, pH 7.2, 0.15 M NaCl, 0.05 % Tween20)
Coupling Reagent:	10 mg/mL EDC** in ice-cooled Binding Buffer, prepared just before the coupling reaction (**EDC: 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride)
Equipment:	Magnetic separator. Vortex tube mixer. Tube rotator.

[Protocol I] For polyclonal antibody or robust monoclonal antibody

1. Suspend the ***Magnosphere™ MS300/Carboxyl*** beads well using Vortex mixer and put 1 mL of the suspension (i.e., 10 mg beads) into a microtube.
2. Place the tube on a magnetic separator for 1 minute (or longer if needed) and remove the supernatant carefully.
3. Add 1 mL of Binding Buffer and suspend the beads by vortexing.
4. Add 100 μg of antibody (100 μL, if antibody was diluted to 1 mg/mL) and suspend the beads by vortexing.
5. Keep rotating the tube with Tube rotator for 30 minutes at room temperature.
6. Add 100 μL of Coupling Reagent and suspend the beads by vortexing.
7. Keep rotating the tube with Tube rotator for 3 hours at room temperature.
8. Remove the supernatant as in step 2.
9. Wash the beads using 1 mL of Washing Buffer and suspend the beads by vortexing.
10. Remove the supernatant as in step 2.
11. Repeat steps 9 & 10 for a total of 3 times.
12. Suspend the beads with a desired buffer suitable for downstream applications and store at 2-8 °C until needed.

[Protocol II] For fragile monoclonal antibody

1. Suspend the ***Magnosphere™ MS300/Carboxyl*** beads well using Vortex mixer and put 1 mL of the suspension (i.e., 10 mg beads) into a microtube.
2. Place the tube on a magnetic separator for 1 minute (or longer if needed) and remove the supernatant carefully.
3. Add 1 mL of Binding Buffer and suspend the beads by vortexing.
4. Add 100 μL of Coupling Reagent and suspend the beads by vortexing.
5. Keep rotating the tube with Tube rotator for 30 minutes at room temperature.
6. Add 100 μg of antibody (100μL, if antibody was diluted to 1 mg/mL) and suspend the beads by vortexing.
7. Keep rotating the tube with Tube rotator for 3 hours at room temperature.
8. Remove the supernatant as in step 2.
9. Wash the beads using 1 mL of Washing Buffer and suspend the beads by vortexing.
10. Remove the supernatant as in step 2.
11. Repeat steps 9 & 10 for a total of 3 times.
12. Suspend the beads with a desired buffer suitable for downstream applications and store at 2-8 °C until needed.

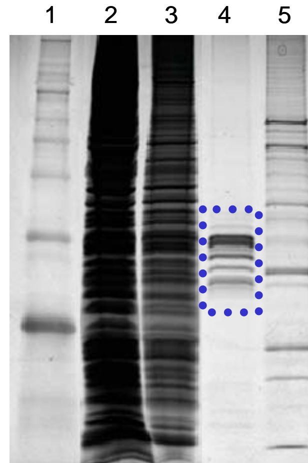
EXPERIMENTAL EXAMPLE

[EXAMPLE I] Immunoprecipitation of 20S proteasome complex from Jurkat cell lysate

Anti-20S proteasome alfa6 monoclonal antibody（Biomol International, L.P, Clone MCP 20) was coupled onto ***Magnosphere™ MS300/Carboxyl*** beads through **Protocol -1**. Immunoprecipitation was performed with the following condition:

- Antibody conjugated beads: 1mg
- Sample: Jurkat cell lysate 100μL (30μg protein)

- IP reaction: 60 minutes at 4 degree C
- Washing: 3 times with 0.5 mL of 20 mM HEPES(PH7.9) + 10v/v% Glycerol + 0.5 M KCl + 0.1% NP-40 + 0.1 mM EDTA and additional one time with 0.5mL of TBS-T
- Elution: Gentle shaking for 10 minutes at room temperature with 20uL of 0.5% SDS (Sodium dodecyl sulfate).
- Detection: SDS-PAGE, Silver stain



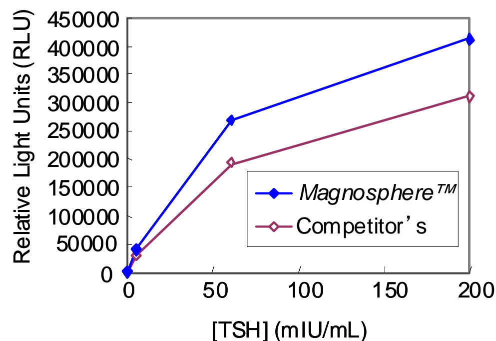
Lane 1 Molecular weight marker
Lane 2 Jurkat cell lysate 40 µg protein
Lane 3 Jurkat cell lysate 4 µg protein
Lane 4 IP product using ***Magnosphere™ MS300/Carboxyl*** beads
Lane 5 IP product using competitor's magnetic beads

By using ***Magnosphere™ MS300/Carboxyl*** beads, subunits (alfa1- alfa7, beta1- beta7) of 20S proteasome complex were isolated with high purity from the cell lysate (lane 4, *inside dotted frame*).

In comparison, the competitor's magnetic beads pulled down many non-specific proteins (lane 5) under the same experimental condition.

[EXAMPLE II] Sandwich ELISA for Thyroid stimulating hormone (TSH)

As a capture antibody, anti-TSH monoclonal antibody (HyTest, Ltd, Clone 10C7) was conjugated onto the ***Magnosphere™ MS300/Carboxyl*** through a protocol II. A 50ug of antibody conjugated bead was reacted with TSH spiked human serum for 30 minutes at 37ºC. The bead was then reacted with secondary antibody labeled with ALP (HyTest, Ltd., Clone:5E8） and chemiluminescence intensity was measured using AMPPD as substrate after washing.



TSH (mIU/mL)	*Magnosphere™*	Competitor's magnetic bead
0	80	150
5	40060	27431
60	268542	191409
200	412546	312716

Noise level, signal intensity at 0mIU/mL, from ***Magnosphere™ MS300/Carboxyl*** was about half against the bead using competitor's and signal intensity at 200mIU/mL was 30% higher than that of competitor's.

IMPORTANT NOTICE

- This product is for research use only and not intended for therapeutic or *in vivo* diagnostic use.
- The specifications of the product may be changed without a notice.
- JSR Life Sciences Corporation does not guarantee that this product will be continuously available.
- JSR Life Sciences Corporation makes no warranties as to this product including, but not limited to, implied warranties of merchantability or fitness for a particular purpose.

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