



Magnosphere™ MS300/Carboxyl

PRODUCT DESCRIPTION

Magnosphere™ MS300/Carboxyl beads are magnetic microparticles designed for immobilization of ligands containing -NH₂ groups for bioseparation. They are ideal for a variety of applications such as enzyme immunoassay, Western blot, and nucleic acid hybridization. The particle surfaces are covered with a JSR proprietary hydrophilic polymer to give the beads their characteristic low non-specific binding and carboxyl groups to provide high affinity.

Features

- Uniform particle size
- Superparamagnetic
- Rapid magnetic responsiveness
- Low non-specific binding
- Surfactant- or oligomer- free
- Stable matrix

Example Applications

Immunoassay, immunoprecipitation, Western blot, nucleic acid hybridization

SPECIFICATIONS

Package volume	4 mL
Solid content in slurry	1% (6 x 10 ⁸ beads/mL approx)
Dispersion media	H ₂ O
Bead diameter	3 μm (micrometer)
Bead magnetite content	20 % approx.
Surface charge density*	10 nmol/mg bead approx.

*Surface charge density = amount of active functional group per 1 mg beads

RECOMMENDED PROTOCOLS: Chemical coupling of antibody on **Magnosphere™ MS300/Carboxyl** beads.

Three examples of recommended protocol of coupling are shown below. The optimum condition may depend on the toughness of the antibody used.

Protocol-1: For polyclonal antibody or robust monoclonal antibody

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.

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. Then, remove the supernatant as in step 2.
4. Add 1 mL of Binding Buffer and suspend the beads by vortexing.
5. Add 100 μg of antibody (100 μL, if antibody was diluted to 1 mg/mL) and suspend the beads by vortexing.
6. Keep rotating the tube with Tube rotator for 30 minutes at room temperature.
7. Add 100 μL of Coupling Reagent and suspend the beads by vortexing.
8. Keep rotating the tube with Tube rotator for 3 hours at room temperature.
9. Remove the supernatant as in step 2.
10. Wash the beads using 1 mL of Washing Buffer and suspend the beads by vortexing.
11. Remove the supernatant as in step 2.
12. Repeat steps 10 & 11 for a total of 3 times.
13. Suspend the beads with a desired buffer suitable for downstream applications and store at 2-8 degrees C until needed.

Protocol-2: For fragile monoclonal antibody

Reagent and equipment requirement

Binding Buffer:	0.1 M MES buffer pH 5.0
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
Equipment:	Magnetic separator. Vortex tube mixer. Tube rotator.

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. Then, remove the supernatant as in step 2.
4. Add 1 mL of Binding Buffer and suspend the beads by vortexing.
5. Add 50 μL of Coupling Reagent and suspend the beads by vortexing.
6. Keep rotating the tube with Tube rotator for 10 minutes at room temperature.
7. Add 100 μg of antibody (100μL, if antibody was diluted to 1 mg/mL) and suspend the beads by vortexing.
8. Keep rotating the tube with Tube rotator for 3 hours at room temperature.
9. Remove the supernatant as in step 2.
10. Wash the beads using 1 mL of Washing Buffer and suspend the beads by vortexing.
11. Remove the supernatant as in step 2.
12. Repeat steps 10 & 11 for a total of 3 times.
13. Suspend the beads with a desired buffer suitable for downstream applications and store at 2-8 degrees C until needed.

Protocol-3: For highly fragile monoclonal antibody

Reagent and equipment requirement

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

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. Then, remove the supernatant as in step 2.
4. Add 1 mL of Binding Buffer and suspend the beads by vortexing.
5. Add 25µL of Coupling Reagent-1 (about 2µmol) and 40 µL of Coupling Reagent -2 (about 2µmol, too) suspend the beads by vortexing.
6. Keep rotating the tube with Tube rotator for 30 minutes at room temperature.
7. Remove the supernatant as in step 2.
8. Wash the beads using 1 mL of Binding Buffer and suspend the beads by vortexing (do not use Washing Buffer containing monoamine such as Tris at this point).
9. Remove the supernatant as in step 2.
10. Add 100 µg of antibody (100 µL, if antibody was diluted to 1 mg/mL) and suspend the beads by vortexing.
11. Keep rotating the tube with Tube rotator for 3 hours at room temperature or overnight at 4 degrees C.
12. Remove the supernatant as in step 2.
13. Wash the beads using 1 mL of Washing Buffer and suspend the beads by vortexing.
14. Remove the supernatant as in step 2.
15. Repeat steps 13 & 14 for a total of 3 times.
16. Suspend the beads with a desired buffer suitable for downstream applications and store at 2-8 degrees C until needed.

EXPERIMENTAL EXAMPLE

Immunoprecipitation of 20S proteasome complex from Jurkat cell lysate

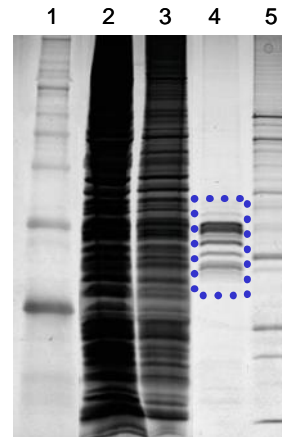
Anti-20S proteasome alfa6 monoclonal antibody (Biomol International, L.P, Clone MCP20) was coupled onto **Magnosphere™ MS300/Carboxyl** beads through Protocol

-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.

STORAGE

Magnosphere™ MS300/Carboxyl is stable for 24 months when stored at 2-8 degrees C. Do not freeze the vial. Vortex the vial or pipette gently up and down to obtain a homogeneous dispersion before use. Following chemical coupling, the product should be stored at 2-8 degrees C. Stability may vary for each of the conjugated product.

IMPORTANT NOTICE

This product is for research use only and not intended for therapeutic or *in vivo* diagnostic use.

REFERENCES

1. HUP0 6th Annual World Congress, Oct 6-10, 2007, p.237

CONTACT INFORMATION

JSR Corporation
URL: http://www.jsr.co.jp/jsr_e/biomedical/index.shtml