Agilent LodeStars 2.7 Carboxyl
Data Sheet

For Research Use Only
LodeStars 2.7 Carboxyl are superparamagnetic particles that are nominally 2.7 µm in diameter. The particle surface is covered with carboxylic acid groups to which ligands can be covalently coupled using well-established chemistries[1]. They are manufactured under stringent control to ensure batch-to-batch reproducibility of their physical and chemical properties. They can be employed as a solid phase in manual and automated assays, and following covalent coupling of specific affinity ligands, can be employed to isolate targets in biological samples including cells, proteins and other biomolecules. LodeStars 2.7 Carboxyl are supplied as a suspension in phosphate buffered saline (PBS) pH 7.4, 0.1% sodium azide.

Chemical and Physical Properties
LodeStars 2.7 Carboxyl are polymeric particles with a microcrystalline ferric oxide component dispersed uniformly throughout the particle. This provides the particles with their superparamagnetic properties, causing them to move rapidly in an applied magnetic field. Also, because no permanent magnetism is retained, they can be fully redispersed once the field is removed.

LodeStars 2.7 Carboxyl particles have a polymer shell that provides two important properties: firstly, it ensures that the internal iron cannot interfere with biological reagents, and secondly, the coating provides carboxylic acid groups for covalent attachment of biomolecules. The polymer surface is highly controlled to provide low non-specific binding of sample components and reduce unwanted non-covalent attachment of ligand that might otherwise be lost from the surface in storage or during a procedure. LodeStars are highly resistant to mechanical stress and reducing conditions, and are stable over a wide pH range.

Size determination of LodeStars is based on optical measurement of close packed arrays. Coated particles are less prone to "tracking" in a Coulter-type counter, but particle sizing by this method may contain errors as the instrument calibration may not take this effect into account. Laser diffraction measurements depend on the accuracy with which the light scattering model reflects the light scattering behavior of the particles being analyzed, and may lead to differences between results obtained by this optical method and Coulter measurements.

Typical Physical Characteristics

Diameter:	2.7 µm
c.v.:	3%
Concentration:	30 mg/mL
Bead Numbers:	~2.3 x 10^9/mL
Iron Content:	20%
Surface Area:	>2 m^2/kg
Magnetic Mass Susceptibility:	>60 m^3/kg
Surface Chemistry:	Carboxylic acid
Antibody Coupling Capacity:	up to 10 µg/mg

INSTRUCTIONS FOR USE

Principle
LodeStars 2.7 Carboxyl is intended as a solid support for a wide variety of applications. The superparamagnetic properties make them ideal for handling and washing. The carboxylic acid surface chemistry is suited to immobilization of a variety of ligands, and the physical and chemical properties of the particles make them versatile in both manual and instrument-based assay and separation systems.

Activation of LodeStars 2.7 Carboxyl is carried out using carbodiimides in methods described below, allowing covalent immobilization of antibody, antigen or other ligands.

Handling and washing of LodeStars is carried out using a magnetic capture stand.

Handling and Washing
It is recommended to wash LodeStars 2-3 times prior to use. The following method is suited to both pre-washing, washing during ligand binding and use of the coated particle.

1. Place the amount of LodeStars required from the storage container into a suitable tube/container and insert into a magnetic capture stand.
2. Allow the particles to collect at the magnet for 2-4 minutes depending on the viscosity of the solution. For very viscous samples, this time may be increased.

3. With the tube still on the magnetic capture stand, remove the supernatant by either decanting or with a pipette, ensuring that the pipette tip does not come into contact with and disturb the magnetically held LodeStars.
4. Remove the tube and LodeStars from the magnetic capture stand and add water or appropriate wash buffer. Note at this point that the LodeStars can rapidly and easily be resuspended with minimal adherence to the tube wall at the magnetic capture point. If the beads are difficult to redisperse, or there are traces of particles adherent to the wall of the tube, consider changing tube type.
5. Repeat as necessary. In most cases three washes are sufficient.

Note: Washing removes sodium azide preservative. For longer term storage of washed beads, consider replenishing with a suitable anti-bacterial agent.

Coating LodeStars with Antibodies

The choice of antibody may influence the separation/assay strategy.

IgM antibodies: These can be covalently immobilized to LodeStars and will generally function efficiently in most applications. Their size and pentavalent structure means they are less affected by steric hindrances. Direct coating of antibodies requires the antibody to be free of carrier proteins. This can be achieved by passing concentrated antibody solution down a column of Sephadex® G-50 (or similar) and collecting the void (excluded) volume. This will contain only high molecular weight material, mainly the IgM.

IgG antibodies: Not all IgG antibodies perform efficiently when coupled directly to a solid phase. The antigen binding sites may be inappropriately orientated with respect to target antigens or cells (steric hindrance), or the antibody can be denatured. Use of a secondary antibody, e.g. sheep anti-mouse IgG acting as a "spacer" can significantly improve efficiency, especially in cell isolation.

Stripping procedures can be used to remove antibody fragments or reaction-degraded proteins to prevent interference. LodeStars are stable for periods in dilute acids, alkali and chaotropic salts, and are resistant to sonication for prolonged periods.

Monoclonal antibodies: A proportion of monoclonals are denatured on adsorption or on coupling to a solid phase, and this must be established by the user. Immobilization onto particles coated with secondary antibodies can be beneficial.

Other ligands: Non-antibody ligands, e.g. lectins, enzymes and proteins in general can be covalently bound to particles and remain functionally active.

Activation and Ligand Coupling

The examples described below are illustrative. They are intended to provide general guidance and should give good results for related biomolecules. However, for each application, optimization of activation and coupling conditions should be carried out, including carbodiimide concentration, ligand concentration, coupling time and temperature.

Antibody Coupling

Coupling antibody to LodeStars employing carboxyl activation with N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (CMC) has been shown to immobilize antibody in a manner that allows it to more effectively bind antigen. This is perhaps due to more favorable antibody orientation on the surface, however the carbodiimide EDC has also been successfully employed. An example of a small-scale coupling of affinity-purified goat anti-mouse IgG-Fc antibody to LodeStars 2.7 Carboxyl is described here.

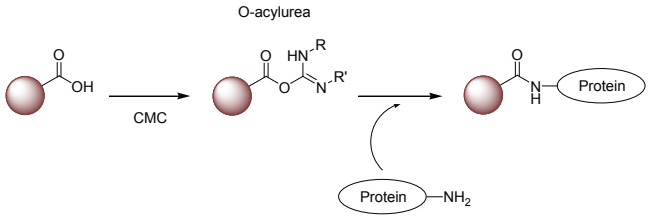

Figure 1. CMC = N-Cyclohexyl-N'-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate.

1. Dispense LodeStars 2.7 Carboxyl (500 µL, approx 1.15 x 10^9 particles) into a 10 mL tube. Collect the particles to the side of the tube by placing it in a magnetic capture stand. Completely aspirate the supernatant with a micropipette.
2. Remove the tube from the magnet and add 2 mL cold deionized water at 4-6 ºC. Vortex the suspension and apply the tube to the magnetic capture stand and aspirate the supernatant. Repeat this water wash twice more, leaving the particles as a pellet following the final wash.
3. Prepare and add immediately 1.6 mL of 5mM CMC solution in deionized water at 4-6 ºC, briefly vortex and mix on a bottle roller for 10 min at this temperature.
4. Apply the tube to the magnetic capture stand again, collect the particles to the side and aspirate the supernatant.
5. Immediately following its preparation, add 1.0 mL 5mM CMC solution in cold 0.3M MES, pH 4.8, briefly vortex and mix on a bottle roller at 4-6 ºC for 30 min.
6. Apply the tube to the magnetic capture stand again, collect the particles to the side and aspirate the supernatant.
7. Wash twice as quickly as possible with 10 mL aliquots of cold 0.1M MES, pH 4.8 at 4-6 ºC, the last time leaving the particles as a pellet.
8. Add 1.0 mL 0.1M MES, pH 4.8, containing purified antibody and briefly vortex and mix on a bottle roller at 4-6 °C for 3 hours. (LodeStars 2.7 Carboxyl will couple up to 10 µg antibody/mg particles, and antibody should be offered to the particles in excess of this capacity).
9. The LodeStars 2.7 Carboxyl particles are applied to the magnetic capture stand and the supernatant aspirated.
10. Wash twice with 10 mL of 0.1M MES, pH 4.8, leaving the particles as a pellet.
11. Block any remaining reactive sites by adding 10 mL 100mM Tris-HCl pH 7.4. Vortex briefly and mix on a bottle roller at room temperature for 1 hour.
12. Wash and store the antibody-coated LodeStars 2.7 Carboxyl in a buffer appropriate for their intended use.

MES = 2-morpholinoethanesulfonic acid, pH adjusted with sodium hydroxide.

The above method may be scaled up or down as required. Antibody coated onto LodeStars 2.7 Carboxyl in this manner may be stable for in excess of 12 months at 2-8 ºC. For long term storage the presence of an anti-bacterial agent is recommended.

Protein Coupling - General Methodology

The surface of LodeStars 2.7 Carboxyl can be activated by converting the carboxylic acid groups to reactive esters using EDC and N-hydroxysuccinimide or N-hydroxysulfosuccinimide. Proteins can be coupled to this reactive surface via their primary amines to form stable amide bonds.

Activation and coupling of proteins with LodeStars 2.7 Carboxyl has been performed in 25mM MES, pH 5 or pH 6, though coupling at other pH in amine-free buffers is possible. The following protocol is therefore illustrative, and optimization should be performed by the user.

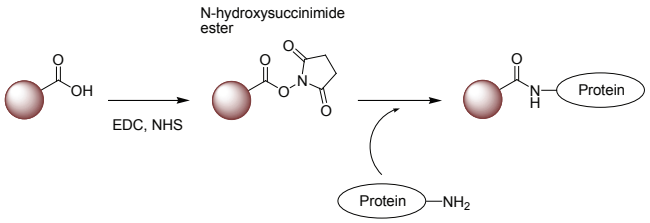

Figure 2. EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, NHS = N-hydroxysuccinimide.

1. Dispense LodeStars 2.7 Carboxyl (500 µL, approx 1.15 x 10^9 particles) into a 10 mL tube. Centrifuge the tube briefly to bring the LodeStars to the bottom and collect the particles to the side by placing the tube in a magnetic capture stand. Completely aspirate the supernatant using a micropipette.
2. Similarly perform 2 x 1.0 mL washes with 0.01M NaOH for 10 min on a bottle roller to leave the LodeStars as a pellet following the final wash.
3. Similarly perform 3 x 1.0 mL washes with deionized water, leaving the LodeStars as a pellet following the final wash.
4. Add 500 µL of a freshly prepared 50 mg/mL solution of N-hydroxysuccinimide in cold 25mM MES, pH 6.0, and vortex mix.
5. Prepare a 50 mg/mL solution of EDC in cold 25mM MES, pH 6.0, and immediately add 500 µL of this solution to the LodeStars suspension and vortex mix.
6. Place the LodeStars on a bottle roller and allow the activation to proceed for 30 min.
7. Centrifuge briefly and apply the tube to the magnetic capture stand and completely aspirate the supernatant.
8. Similarly, quickly perform 2 x 10 mL washes with cold 25mM MES, pH 5.0, using vortexing to resuspend the LodeStars and magnetic separation each time to leave them as a pellet.
9. Add 600 µL of 25mM MES, pH 5.0, followed by 600 µL of a 1 mg/mL solution of protein in the same buffer and vortex, and allow the coupling to proceed by mixing on a bottle roller for 3 hours at room temperature.
10. Apply the tube to the magnetic capture stand and aspirate the supernatant. Add 10 mL 100mM Tris-HCl, pH 7.4. Vortex briefly and mix on a bottle roller for 1 hour to block any remaining reactive sites.
11. Transfer the LodeStars to a buffer appropriate to their application.

Reference

[1] 'Bioconjugation' Protein Coupling Techniques for the Biomedical Sciences, M Aslam, A Dent. Macmillan Reference Ltd, London (1998). ISBN 0-333-583752.

Shipping and Storage

LodeStars 2.7 Carboxyl are shipped at ambient temperature and should be stored at 2–8 °C.

Limitations

For research use only: not for use in human diagnostic or therapeutic procedures.

Contains sodium azide: do not pipette by mouth. Sodium azide is reactive with copper and lead pipes to form explosive compounds. Flush plumbing well with water when disposing to prevent build-up.

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Ordering Information

Product	Part No.
LodeStars 2.7 Carboxyl 2 mL @ 30 mg/mL	PL6727-0001
LodeStars 2.7 Carboxyl 10 mL @ 30 mg/mL	PL6727-0003
LodeStars 2.7 Carboxyl 100 mL @ 30 mg/mL	PL6727-0005

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