



Leading the Way

LodeStars™ 2.7 Streptavidin

For research use only

Product	Part No.
LodeStars 2.7 Streptavidin 2ml@10mg/ml	6727-1001
LodeStars 2.7 Streptavidin 10ml@10mg/ml	6727-1003
LodeStars 2.7 Streptavidin 100ml@10mg/ml	6727-1005
PL-MCS2 Magnetic Capture Stand <i>for use with 1 or 2 tubes from 10-50ml</i>	6700-0001
PL-MCS12 Magnetic Capture Stand <i>12 magnetic positions for 1.5 and 2.0ml tubes</i>	6700-0002
PL-MCS96 Magnetic Capture Stand <i>for use with regular and deep 96 well plates</i>	6700-0003

LodeStars magnetic particle-based separation technology is a family of superparamagnetic particle products from Polymer Laboratories. LodeStars products have applications across numerous areas of bioscience research, diagnostics and therapeutics, as well as in the development of new products in molecular medicine.

LodeStars™ 2.7 Streptavidin

Typical Physical Characteristics

Diameter	2.7µm
Concentration	10mg/ml
Bead Numbers	~8x10 ⁸ /ml
Iron Content	20%
Surface Area	>2m ² /g
Magnetic Mass Susceptibility	>60m ³ /kg
Surface Chemistry	Streptavidin
Biotin Binding Capacity	>900 pmoles/mg
Biotinylated IgG Binding Capacity	>4.0g/mg

LodeStars 2.7 Streptavidin 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 chemical groups for covalent attachment of the streptavidin. 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.

Size measurements for LodeStars are 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

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scattering model reflects the light scattering behaviour of the particles being analysed, and may lead to differences between results obtained by this optical method and Coulter measurements.

INSTRUCTIONS FOR USE

Principle

LodeStars 2.7 Streptavidin is intended as a solid support for a wide variety of applications. Their superparamagnetic properties make them ideal for handling and washing. The streptavidin surface is suited to immobilization or capture of a variety of biotinylated ligands, and the physical and chemical properties of the particles make them versatile in both manual and instrument based assay and separation systems.

LodeStars 2.7 Streptavidin are ready to use in a procedure.

Handling and washing of LodeStars is carried out using a PL-Magnetic Capture Stand (PL-MCS). LodeStars are highly resistant to mechanical stress and stable over a wide pH range. Streptavidin is a relatively stable protein, but care must be taken to avoid denaturation through chemical, physical or biological degradation.

PRODUCT DESCRIPTION

LodeStars 2.7 Streptavidin are monodisperse superparamagnetic particles nominally 2.7µm in diameter and coated with streptavidin, which is a 56kDa four-subunit protein that binds with exceptionally high affinity to biotin ($K_D = 10^{-15}$ M). Biotin is a small molecule that can be used to label biological molecules, e.g. antibodies, which can then be captured with LodeStars 2.7 Streptavidin.

LodeStars 2.7 Streptavidin are manufactured under stringent controls 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 used to isolate targets in biological samples including cells, proteins and other biomolecules. LodeStars 2.7 Streptavidin are supplied as a suspension in phosphate buffered saline (PBS), pH 7.4, 0.1% sodium azide.

Chemical and Physical Properties

LodeStars 2.7 Streptavidin are polymeric particles with a microcrystalline ferric oxide component dispersed uniformly throughout the particle. This provides 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.

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 following ligand binding and washing during use with water or appropriate buffers.

1. Place the LodeStars required from the storage container into a suitable tube/container and insert into the PL-MCS.
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 PL-MCS, 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.
- Remove the tube and LodeStars from the PL-MCS and top up with fresh wash buffer, e.g. PBS. 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 particles are difficult to redisperse, or there are traces of particles adherent to the wall of the tube, consider changing tube type.
 - 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.

Working with LodeStars and Biotinylated Antibodies

To bind to LodeStars 2.7 Streptavidin, ligands must be biotinylated.

Biotinylation

There are several methods for labelling biomolecules by the covalent attachment of biotin. A considerable number of biotinylation reagents are sold by manufacturers e.g. Pierce, though in practice, the choice of reagent will be restricted by the target moieties available in the biomolecule. In proteins, the most common targets are primary amines which react with biotinylation reagents incorporating reactive esters, e.g. N-hydroxysuccinimide esters.

Sulfhydryl groups are commonly employed as alternative target moieties that can be introduced into proteins by reduction of disulphide bridges in the protein, or by reaction of amines with Traut's reagent or N-succinimidyl S-acetylthioacetate.

Biotinylation of antibodies can be optimized so that binding of the biotin label to streptavidin on the particle causes minimal steric hindrance of the subsequent binding of antigen to the antibody. To achieve this, structures on the antibody distant from the antigen binding sites, e.g. carbohydrate structures on the Fc region, can be labelled with biotin. It is also advantageous to employ a spacer group between the biotin moiety and the biomolecule to minimize steric hindrance of the biotin binding to streptavidin, and maximize flexibility of the biotinylated biomolecule once bound to the streptavidin on the particle. Many biotinylation reagents incorporate such spacer structures.

Please refer to the references below for more extensive information on biotinylation reagents and methods.

Biotinylated Antibody Binding

The binding of biotinylated antibody to LodeStars 2.7 Streptavidin is achieved by simply mixing a solution of the antibody with the LodeStars 2.7 Streptavidin, and washing away unbound excess antibody. The very high affinity of the biotin-streptavidin interaction ensures rapid and essentially permanent binding. There are however, factors which will affect the resulting product with regard to how much antibody is immobilized, how effective it is, and the degree of LodeStars 2.7 Streptavidin aggregation induced by multiple biotins on the antibody. These include primarily, particle and biotinylated antibody concentration, and the number of biotins and their location on the antibody. The user is strongly recommended to optimise these factors in each application.

As an example, coating of LodeStars 2.7 Streptavidin with biotinylated antibody has been performed by adding a suspension of the particles at 1mg/ml in 0.1% Tween 20, 0.1% BSA, PBS pH 7.4, 0.05% sodium azide with an equal volume of biotinylated antibody solution (40µg antibody/ml) in the same buffer. Typically, binding has been over a period of 30 minutes with gentle mixing, and the particles are then washed extensively with the same buffer or another buffer appropriate to their use.

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

Additional Materials Needed

PL-Magnetic Capture Stands (PL-MCS)

PL-MCS2: for use with 1 or 2 tubes from 10-50ml

PL-MCS12: 12 magnetic positions for 1.5 and 2.0ml tubes

PL-MCS96: for use with regular and deep 96 well plates

References

'A Laboratory Guide to Biotin-labeling in Biomolecule Analysis', BioMethods, Vol. 7. Series editors T Meier and F Fahrenholz. Published by Birkhauser Verlag, Basel, Switzerland (1996). ISBN 3-7643-5206-X (Basel).

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

Shipping and Storage

LodeStars 2.7 Streptavidin 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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