A Versatile Biotoool
for Bioseparation, Biopurification and Biodetection

Bio-Estapor microspheres

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
More than 20 years experience with superparamagnetic microspheres

Estapor® Microspheres, one of the world’s leading manufacturers of microspheres, develops, produces and distributes worldwide more than 200 different types of microspheres. Magnetic, white, dyed or fluorescent microspheres are key components for reagent producers in clinical diagnostics, life sciences, food and environment industries.

Certified ISO 9001: 2000 and ISO 14001, our quality system and our patented processes are strictly controlled and reviewed, well-established and stringently validated resulting in consistent quality.

Merck Chimie SAS introduces its new Bio-Estapor product line. This new line includes Streptavidin, anti-mouse IgG, anti-human IgG, Protein A and Protein G coated microspheres, allowing researchers to optimize any Bioseparation, Biopurification or Biodetection applications, without sacrificing sensitivity and ease of use.
The Added Value of Magnetic Microspheres

The use of magnetic microspheres is a well established alternative to conventional chromatography resins in automated high throughput protocols replacing centrifugation, organic solvents and filtration by simple and rapid magnetic separation.

Bio-Estapor microspheres combine these advantages with the unique features like uniform size, high magnetite content and nonporous surface allowing them to migrate very quickly in magnetic fields (figure 1) while binding target molecules with a low unspecific adsorption.

**Figure 1.** Isolation of Bio-Estapor microspheres in microtubes or in 96-well microtitre plate.

An Innovative Technology for Precise Assay Requirements

Biocoated microsphere requirements for Bioseparation of human cells (1) or bacteria (2), Biopurification of immunoglobulins or recombinant proteins (3) are different from those used in Biodetection for immunoassays (4) or ELISA (5).

According to this evidence and in anticipation of the researcher’s expanding needs for biotools to meet precise assay requirements, our new line integrates several innovative technologies. They include: sizes (0.3 µm, 1.0 µm and 2.6 µm), surface properties (hydrophilic or hydrophobic) and ferrite contents (30-50%).

Our 0.3 µm Bio-Estapor microspheres provide a high surface area, guaranteeing a maximum binding capacity and show almost no sedimentation, but they will separate slowly especially in a complex or viscous matrix.

In comparison and with a high magnetophoretic mobility, our 2.6 µm Bio-Estapor microspheres will separate rapidly in a complex liquid (8) but they have less surface area and will not remain very long in solution during the binding process.

The 1.0 µm Bio-Estapor microspheres are often the best compromise and combine efficient magnetophoretic mobility, high surface area and a low sedimentation rate. This is probably why today the 1 µm size is the gold standard for chemiluminescent immunoassays (4,5,6).

Working on a hydrophilic or lipophilic surface may help to increase signal to noise ratio.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Advantages</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superparamagnetic microspheres</td>
<td>Easy and rapid magnetic separation</td>
<td>Easy automation and time saving</td>
</tr>
<tr>
<td></td>
<td>Simple resuspension</td>
<td>Better reproducibility</td>
</tr>
<tr>
<td></td>
<td>Higher surface area than coated tubes or ELISA</td>
<td>High sensitivity for low concentration analytes</td>
</tr>
<tr>
<td>Small size</td>
<td>High surface area</td>
<td>Increased sensitivity and capture</td>
</tr>
<tr>
<td></td>
<td>Low sedimentation rate</td>
<td>Less beads to be used with no loss in performance; cost saving</td>
</tr>
<tr>
<td>Large size</td>
<td>High magnetophoretic mobility</td>
<td>Better kinetic and shorter analytical time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher recovery in a complex matrix or viscous liquid</td>
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Streptavidin Coated microspheres

The use of streptavidin microspheres in Bioseparation, Biopurification or Biodetection is based on the very high affinity of streptavidin for biotin labelled molecules.

The streptavidin-biotin interaction is very strong (Kd = 10^{-15} M) and allows the separation, purification and detection of biotinylated molecules under different pH conditions (3-10), buffers (0.01-3.0 M salt), and detergents such as SDS or Tween 20 without effect. Our streptavidin coated magnetic microspheres provide an efficient solid-phase for separating or purifying many types of biotinylated biomolecules including: antigens, antibodies or nucleic acids from different sources such as blood, sera, tissues and food. Recently, our Streptavidin coated microspheres have been used in a electrochemical sensing of aptamer-protein interactions (9) and in a rapid detection of Methicillin-resistant staphylococcus aureus (2).

**Figure 2**: Application of Bio-Estapor streptavidin microspheres – mRNA Biopurification.

**Other Applications**

Bacteria concentration, human or animal cell separation, proteomics, PCR product purification.
Anti-Mouse IgG and Anti-Human IgG Coated microspheres

The bioconjugation of magnetic microspheres with antibodies combines the properties of the microspheres and the specific and selective recognition ability of the antibodies to antigens. These antibody coated microspheres offer several advantages to the user. The secondary antibody acts as a spacer and helps to reduce possible steric hindrance. It could allow the attachment of an entire primary antibody or antibody fragments like Fab or F(ab)\(_2\) by a simple bioconjugation and eliminate the use of chemical coupling which could impact their bioactivity. Monoclonal antibodies are recommended for coupling and this option has been used to develop a direct immunoassay for cobalamin bound to holo-transcobalamin in serum (4).

**Figure 3:** Application of Bio-Estapor anti-human IgG – Antigen Biodetection by immunoassays.

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**Other Applications**

Immunoprecipitation, cell separation, proteomics.
Protein A and Protein G coated microspheres are used for capturing immunoglobulins from a variety of samples like: plasma, cell culture supernatants or other aqueous solutions. The loaded particles with Protein A-IgG or Protein G-IgG complexes can be used for immunoprecipitation of target antigens.

Our Protein A and Protein G Bio-Estapor microspheres, have been used for Antibody and Fc-fusion protein purification from cell culture supernatant (3). The binding activity of the purified proteins was intact, with similar dose responses for proteins purified with either magnetic microspheres or Protein A Sepharose resin (7). In terms of cost, our Protein A and Protein G coated microspheres are reusable for at least 10 times.

For development of immunossays, Protein A or Protein G coated microspheres allow the bioattachment of IgG through its Fc portion and eliminate the use of chemical coupling.

**Figure 4:** Application of Bio-Estapor protein A and G- IgG purification.

1. **CAPTURE**
   - Protein A/G coated Beads
   - Cell culture supernatant

2. **MAGNETIC SEPARATION**
   - Protein A/G
   - Flow through

3. **ELUTION**
   - Protein A/G

4. **MAGNETIC SEPARATION**
   - Protein A/G
   - Purified Abs

**Other Applications**

Immonoassays, cell separation, proteomics.
Range

<table>
<thead>
<tr>
<th>Designation</th>
<th>Ligand</th>
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<tbody>
<tr>
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<td>Streptavidin</td>
<td>Small</td>
<td>80 380 097</td>
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<td>BE-M06/1,0</td>
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<td>BE-M06/1,0</td>
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<tr>
<td>BE-M07/1,0</td>
<td>Protein G</td>
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<td>80 380 157</td>
</tr>
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</table>

Hydro = hydrophilic  –  Lipo = lipophilic  –  Fus = fusion protein

We offer 5 standard pack sizes: 1 ml, 2 ml, 5 ml, 10 ml and 50 ml at 1% solid content (10mg/ml). Larger pack sizes, bulk quantities and custom Bio-Estapor are available upon simple request.

References


We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

All products are not intended for use as in–vitro diagnostics in terms of European Directive 98 /79 / EC. They are for research purposes only, for investigating in–vitro samples without any medical objective.

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