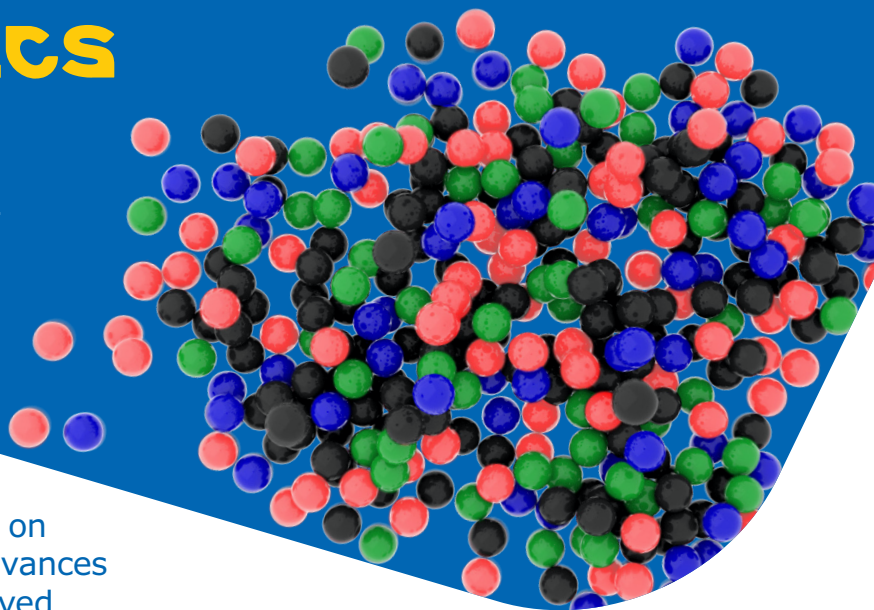


NEWS on diagnostics

MILLIPORE
SIGMA

2020 Volume 4

Advances in microsphere technology for diagnostic applications



Welcome to the latest edition of News on Diagnostics. This volume discusses advances in microsphere technologies for improved diagnostic assay performance.

For over forty years, Estapor® Microspheres has been a leading brand of polymeric supports for *in vitro* diagnostics, life sciences and biotechnology applications. We develop, manufacture, and provide a broad portfolio of Estapor® microspheres worldwide.

Microspheres and Nanospheres in Diagnostic Immunoassays

Since Singer and Plotz first described the rheumatoid factor (RF) test in 1956, which is based on the agglutination of white microspheres, many tests and assays using this technology have been developed by numerous companies worldwide. Today microspheres are commonly used in assays for the detection and/or quantification of hormones, antibiotics, autoimmune diseases, microbial and viral infections among other applications.

Using microspheres in lieu of a coated tube or ELISA plate offers many advantages including improved assay performance and flexibility for use in many different detection formats. Microspheres have a larger surface area, yielding faster kinetics and lower detection limits. Their surfaces can also be functionalized with different surface chemistries, providing better protein coverage and antibody orientation.

Estapor® Microsphere Assay Applications

Lateral Flow Assays – IVD test strips, based on the principles of immunochromatography, exist for a wide array of target analytes. This technology has advanced rapidly since the first human gonadotropin (hCG) tests. Today, there is an extensive offering of commercially available tests with Estapor® microspheres, the top choice for many. As part of our lateral flow microsphere offering, we provide dyed microspheres for simple qualitative or semi-quantitative readings as well as fluorescent and magnetic microspheres for quantitative assays.

Chemiluminescent Immunoassays (CLIA) – Many factors must be considered before the development of an immunoassay. One critical consideration is the sensitivity; what level of analyte do you need to detect? If your analyte of interest is <10 ng/mL, you may need to consider a CLIA over the typical ELISA as it offers increased sensitivity and full automation for high-throughput screening. To support your CLIA development needs, we offer a comprehensive portfolio of superparamagnetic microspheres.

Immunoturbidimetric Assays (IT) – When serum proteins are not detectable with classic chemistry methods, IT assays are an important tool in your kit. IT assays leverage simple antigen-antibody reactions to form aggregate complexes which are optically detected by a photometer. Our brand new Estapor® IT microspheres are coming soon.

Lateral Flow Assay

Lateral flow assays are among the most ubiquitous and versatile diagnostic tests on the market today. Despite their prevalence in the market, these assays can be complex to develop with multiple considerations involved. These assays exist at the intersection of biology, physics, chemistry, and engineering and require understanding of each of these disciplines for successful development.

Most lateral flow assays are designed in one of four formats: sandwich, competitive, inhibition, or serum assays. To learn more about the role our Estapor® microspheres play in lateral flow assay design please refer to our brochure “Estapor® Microspheres For Lateral Flow” at EMDMillipore.com/estapor

Beyond the assay format, there are many factors that need to be considered and controlled, including material

selection, reagent selection, sample flow control, desired signal intensity, and manufacturing processes. To fully address each of these factors, there are a variety of contributing components to be assessed.

One of the most important decisions during the assay development process is around the size of the detector particle you will use and the speed of the lateral flow membrane. There is an inverse relationship between run speed and assay sensitivity, with larger microspheres running slowly and smaller microspheres moving through the assay more quickly. When selecting the largest available microsphere, the detector particles will have more time to interact with the target analyte, possibly resulting in a more sensitive assay in exchange for longer run times. Balancing these two factors will be at the core of your assay design.

NEW! To learn more about the lateral flow assay development process, download our newly released application note “Development of a Quantitative Lateral Flow Test using Estapor® Europium Microspheres” visit at EMDMillipore.com/Estapor

Dyed Microspheres

Dyed microspheres are best suited for assays where the primary goal is simply detecting the presence or absence of the target analyte. These qualitative assays are often the simplest to design and don’t require specialized equipment to read the result. Dyed microspheres can also be used in semi-quantitative assays where the color presented can be compared with a reference to give a rough idea of the quantity of analyte present.

To support your qualitative lateral flow development needs, we offer four different colors of dyed microspheres (black, blue, red, and green) in various different sizes both with and without surface functionalization. For a full listing visit

EMDMillipore.com/estapor-dyed



Figure 3 Lateral flow tests using Estapor® Dyed Microspheres (reference: K1-030 blue for the control, K1-030 red and K1-030 green for the analytes) and Millipore Hi-Flow™ membrane.

Estapor® XC Microspheres

In recent years, there has been a significant shift towards the development of quantitative lateral flow assays. Utilizing fluorescently-labeled detector particles, these assays allow clinicians to precisely and accurately determine how much of the target analyte is present in the sample. Used in millions

of rapid diagnostic assays worldwide, our XC microspheres offer consistency and ease of use in quantitative lateral flow assays, deeming them a convenient and reliable choice. See our product offering at EMDMillipore.com/estapor-fluorescent

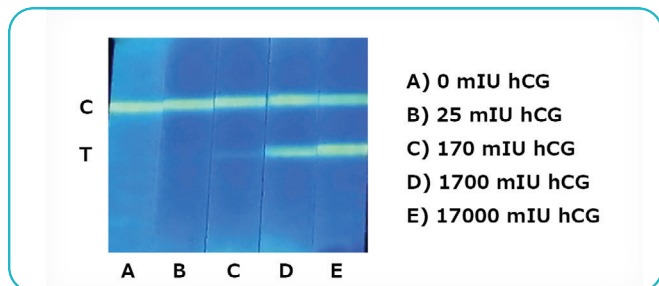


Figure 4 Qualitative image of Human Chorionic Gonadotropin (hCG) lateral flow test strips utilizing XC fluorescent microspheres viewed under UV light. Key: C= Control line, T=Test line

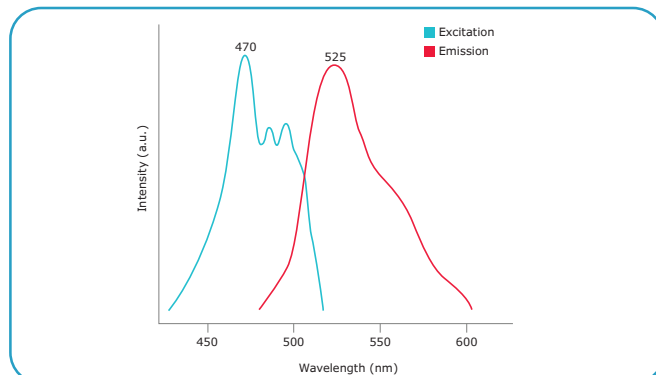


Figure 5 Schematic of the Excitation and Emission profile of XC. Max excitation and emission wavelengths indicated on each peak. *Note: XC is well suited to the 488nm wavelength of the Argon laser.*

NEW! Estapor® Europium Microspheres

The launch of our new Estapor® Europium Microspheres aims to enhance the sensitivity of your assays and expand the possibilities in lateral flow development. As the latest part of our comprehensive lateral flow portfolio, we have developed these microspheres in order to provide several advantages:

- Significantly improved quantitative lateral flow assay sensitivity
- Reduced background fluorescence

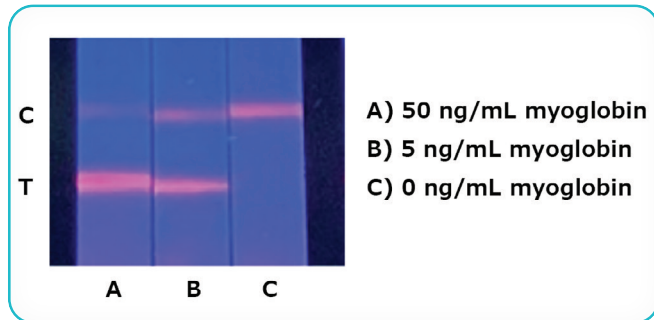


Figure 6 Myoglobin lateral flow test strips utilizing 300nm Europium microspheres viewed under UV light. Key: C= Control line, T= Test line

- Simplified assay readability and easier to quantify
- Longer Stokes shift than traditional fluorescent labels
- Enhanced fluorescent quantum yield facilitating a low detection limit
- Functionalized with a carboxylated surface for easy protein conjugation
- Range of sizes available; choose the best for the desired target analyte

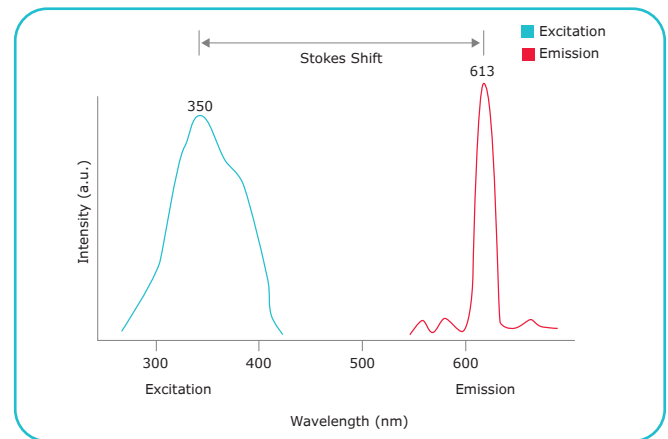


Figure 7 Schematic of the Excitation and Emission profile of Europium. Max excitation and emission wavelengths indicated on each peak. *Note: the very narrow emission peak, as well as the large Stokes shift of the fluorophore.*

Want to test the advantages of Estapor® Europium microspheres for yourself?
 Request a sample at: EMDMillipore.com/Estapor-europium-sample

Chemiluminescent Immunoassays (CLIA)

Leveraging the combination of chemiluminescence and immunochemical binding, CLIA is an excellent choice of diagnostic test due to their high sensitivity and specificity, their versatility, and ease of use.

When choosing microspheres for a CLIA assay, there are several considerations to be aware of:

- Size
- Composition
- Coating Procedure
- Antibody Selection

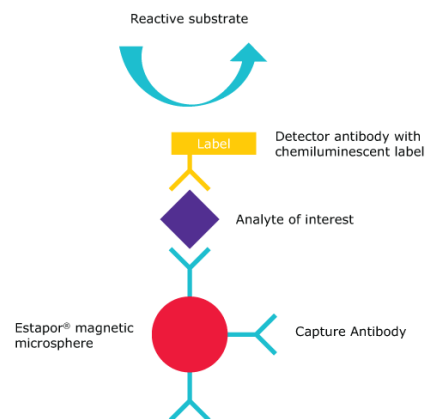


Figure 8 Estapor® magnetic microspheres serve as the foundation upon which the chemiluminescent reaction occurs, while allowing for easy separation and washing.

NEW! To learn more about the CLIA development process, download our newly released application note "Development of a chemiluminescent immunoassay (CLIA) using Estapor® magnetic microspheres" at EMDMillipore.com/Estapor

Magnetic Microspheres

As the core material in most CLIAs, magnetic microspheres serve to both capture the analyte of interest and protect the bound complex during the various washing steps of a standard CLIA protocol. Depending on the analyte of interest, it may be desirable for the microsphere to have a carboxyl coating or to be coated with a different conjugate to aid in antibody binding. Regardless of your need, our comprehensive portfolio of magnetic microspheres has the options you are looking for. Each with its own advantages, our portfolio includes:

- Estapor® Carboxylated Hydrophilic Magnetic Microspheres
- Estapor® Carboxylated Hydrophilic Magnetic Nanospheres
- Estapor® Hydrophobic Magnetic Microspheres
- Encapsulated Estapor® Magnetic Microspheres
- Bio-Estapor® Magnetic Microspheres

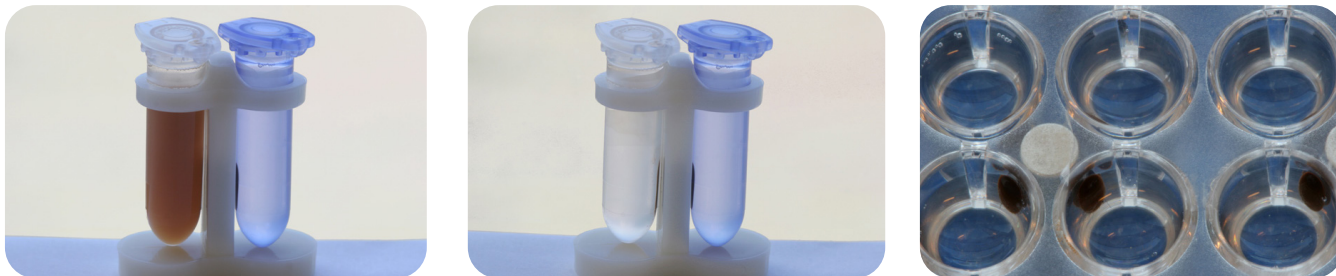


Figure 9 Magnetic separation of Estapor® Magnetic Microspheres is compatible with small tubes in manual format (left and center), 96-well microtiter plates (right), as well as many automated platforms on the market.

For more on the benefits and best use of each of these magnetic microspheres, visit EMDMillipore.com/estapor-magnetic

Production Capabilities and Associated Services

Polymer microspheres are critical raw materials for reagent and kit manufacturers. New lots of materials need control and validation, which require time and money. For some IVD companies, a small amount of material on the order of a few grams will satisfy their annual production needs; for others, several kilograms of raw material may be needed.

In 90% of cases, standard microsphere and nanosphere products should be able to fulfill your manufacturing needs. However, our custom development services may be utilized to support demanding applications. Using state-of-the-art technology, our scientists can provide customized microspheres that will meet specific needs and requirements.

On-Demand Resources

We are committed to communicating new developments with our customers. As a result, we have webinars available on demand.

An introduction to our portfolio can be found at SigmaAldrich.com/EstaporWebinar

In conjunction with a collaborative partner, Anteotech, we are delighted to present a webinar on our new Europium microspheres. Please visit SigmaAldrich.com/anteotechwebinar to access this information.

If you're interested in learning more about how Estapor® magnetic microspheres can be used to aid in the separation process of CLIA protocols, please visit SigmaAldrich.com/BiomagneticSeparationWebinar to review our recent webinar hosted by Dr. Lluís Martínez of SEPMAG.

Want to learn more?

Additional reading material as well as product information can be found online at EMDMillipore.com/estapor