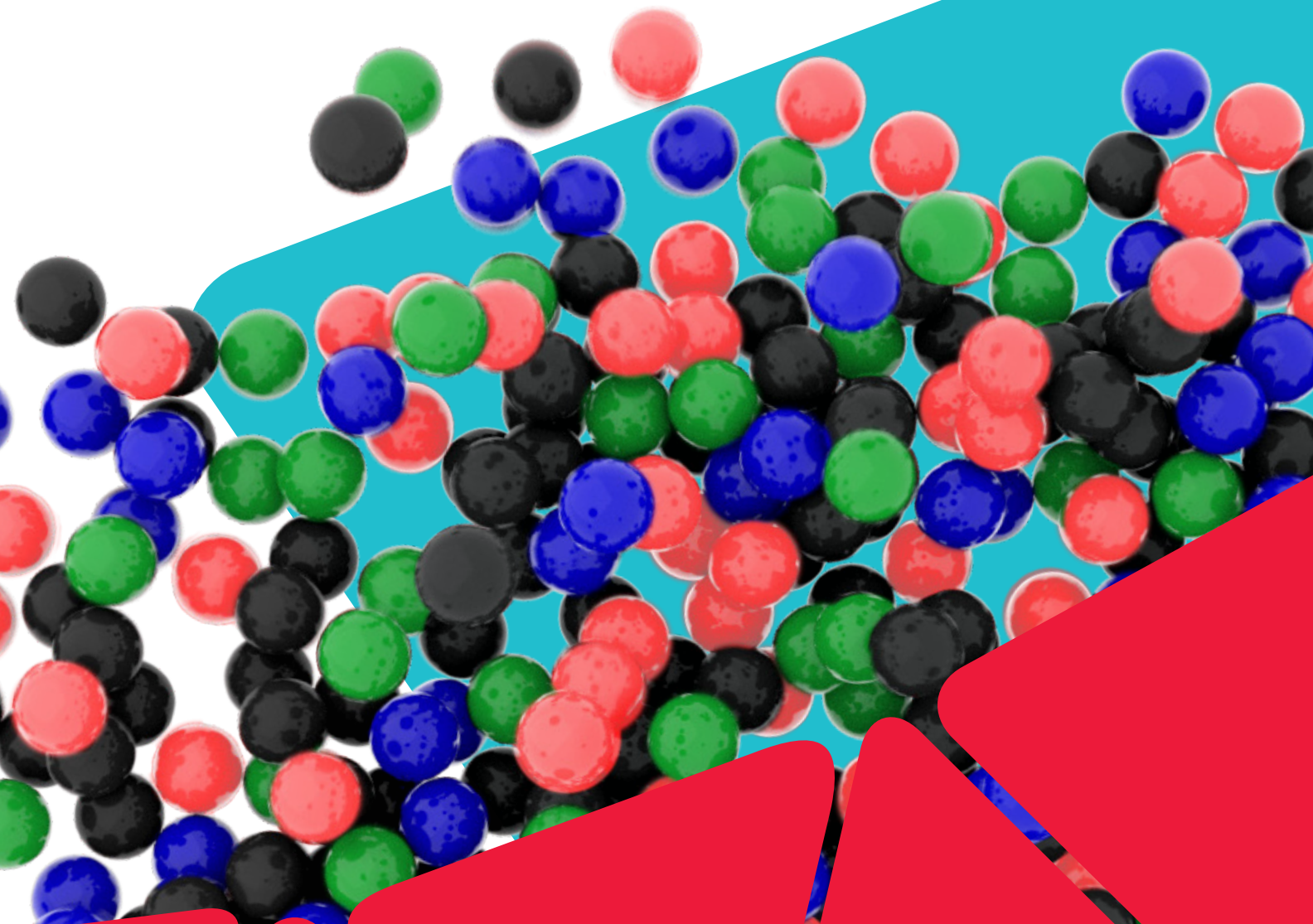
Estapor® Microspheres for Lateral Flow

Colored, Fluorescent and Magnetic Microspheres for Qualitative and Quantitative Lateral Flow assays



Sigma-Aldrich®

Lab & Production Materials

Estapor® Microspheres for Lateral Flow

Committed to providing our worldwide partners with the highest quality microspheres, service, and technical expertise; we offer the components and support for optimizing your diagnostic manufacturing needs.

Offering quality, reliability and choice —
We are your global partner.

For the last 25 years, Estapor® microspheres have been the top choice for many lateral flow diagnostic test manufacturers. Used in millions of assays made by internationally known brands, our microspheres are adapted to both manual and automated platforms. They are highly characterized, customizable, and designed with key features to ideally meet the needs of IVD manufacturers.

Key Features & Advantages

Reproducibility:

Estapor® microspheres are polystyrene-based which affords rigidity, dimensional stability, size uniformity and exceptional lot-to-lot reproducibility.

Supply Reliability:

Our state of the art manufacturing facility produces microspheres from gram to industrial (kg) scale facilitating a robust and reliable supply.

Choice:

We are committed to offering the right bead for the right application. Our Estapor® 'Lateral Flow' portfolio offers various sizes, four different colors, two fluorescence choices and two magnetic options. 20 different microspheres, all optimized for your lateral flow needs.

Established & Proven:

Supplying a wide range of microspheres to lateral flow manufacturers for over 25 years, Estapor® offers a recognized and established portfolio of microspheres with proven success.

Technical Support:

Estapor® customers benefit from the extensive knowledge of our dedicated technical team and long-standing microsphere manufacturing experience.

Flexibility:

Estapor® microspheres are suitable for many types of lateral flow assays such as Sandwich, Competitive, Inhibition and Serological. They are used extensively by our global partners to detect a wide range of target analytes across a range of biological sample types.

Enhanced Performance:

Estapor® lateral flow microspheres are easily dispersed allowing for efficient washing, reduced background and low non-specific binding. Our microspheres do not experience dye leaching, display maximum color brilliance and exhibit stable surface properties so you get the performance you expect every time!

Estapor® Microspheres for Lateral Flow

We have been a key partner and supplier to rapid diagnostics manufacturers since the industry's beginning. 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 colored microspheres for simple qualitative or semi-quantitative readings as well as fluorescent and magnetic microspheres for quantitative assays.

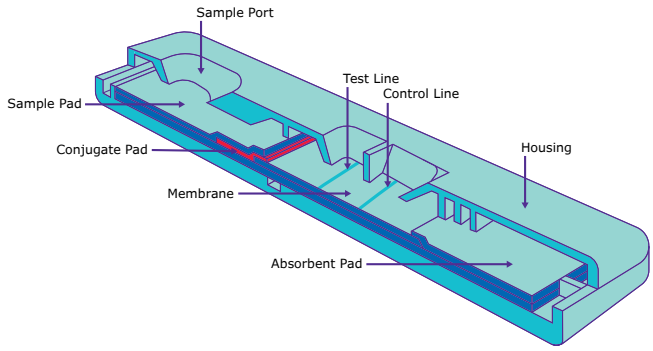
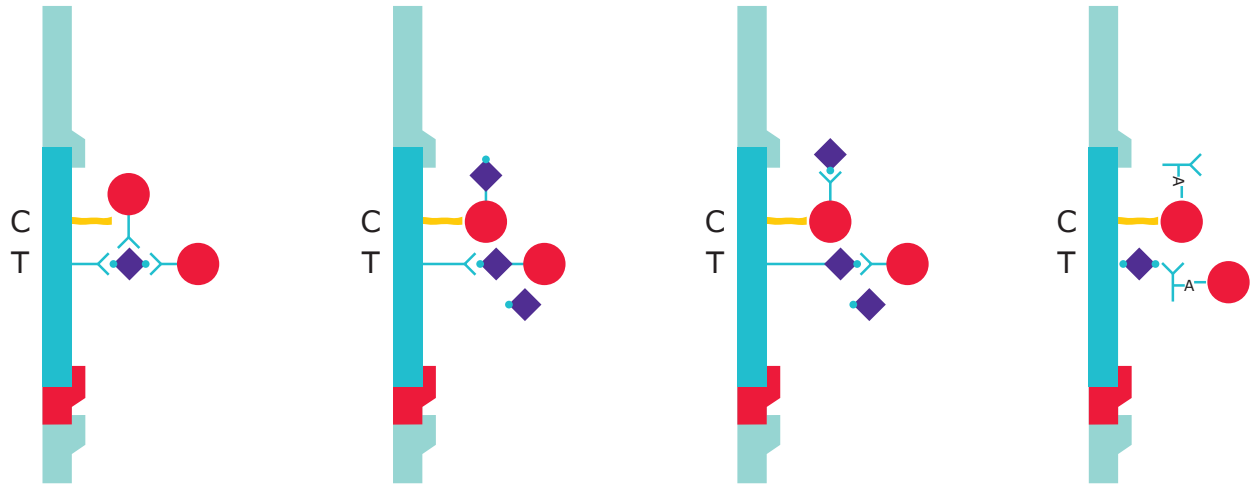


Fig. 1 Lateral flow test strip design



Sandwich assays: The detection particle – a microsphere coated with the antibody of interest – is temporarily immobilized in the conjugate pad. When a sample passes through the conjugate pad, the target analyte binds to the conjugated antibody and forms a labeled antibody conjugate/analyte complex. This complex binds to the test line (T) antibody; and the analyte becomes sandwiched between conjugated and primary antibodies, forming a labeled antibody conjugate/analyte/primary antibody complex.

Competitive assays: The target analyte is conjugated to the detection particle and temporarily immobilized in the conjugate pad. The sample containing the target analyte is applied to the sample pad. The conjugated particles and the analyte both move from the conjugate pad through the membrane. They compete for binding to the test line (T) antibody. The absence of color at the test line indicates a positive result.

Inhibition assays: Like a competitive assay, the absence of a line indicates a positive result. In this case, the antibody is conjugated to the detector particle, and target analyte is immobilized on the membrane. The antibody conjugated to the detector particle is inhibited from binding to the immobilized analyte in the presence of excess target analyte in the sample.

Serological assays: This assay detects antibodies in serum or whole blood. The analyte is immobilized on the membrane. Specific antibodies present in the sample bind to the analyte immobilized on the membrane and are non-specifically recognized by Protein A (A) conjugated to the detector particles. The presence of color at the test line indicates a positive result. A quantitative result can be achieved if fluorescent particles are used along with an electronic reader.

Key: C= Control Line, T= Test Line,
◆ = target analyte, ● = microsphere, Y= antibody

Fig. 2 Estapor® Microspheres are optimized for use in various types of qualitative and quantitative lateral flow tests

Estapor® Colored Microspheres for Lateral Flow

Our colored microspheres are best suited for assays where the primary goal is simply detecting the presence or absence of the target analyte. Choose the desired combination of surface functionalization, color and size as outlined below to find the best microsphere for your application.

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

Table 1: Estapor® Colored Microspheres

Product	Color	Diameter (µm)	Surface COOH (µeg/g)	Cat. No.
K-030 Black	●	0.270 -0.325	None	39442088
K1-030 Black	●	0.270 -0.330	200 - 500	80380143
K1-050 Black	●	0.450 -0.530	1 - 10	80380238
K-030 Blue	●	0.270 -0.325	None	39428080
K1-030 Blue	●	0.270 -0.330	200 - 500	80380065
K1-050 Blue	●	0.450 -0.530	1 - 10	39544001
K-030 Red	●	0.270 -0.325	None	39482086
K1-030 Red	●	0.270 -0.330	200 - 500	80380011
K1-050 Red	●	0.450 -0.530	1 - 10	39545001
K-030 Green	●	0.270 -0.325	None	80380004
K1-030 Green	●	0.270 -0.330	200 - 500	39450086
K1-050 Green	●	0.450 -0.530	1 - 10	39546001

Estapor® Fluorescent Microspheres for Quantitative Lateral Flow

Estapor® XC Microspheres

Our XC fluorescent microspheres are best suited to quantitative lateral flow assays. With their monodispersity and uniformity, creating a standard curve for analyte quantification is easy and reliable.

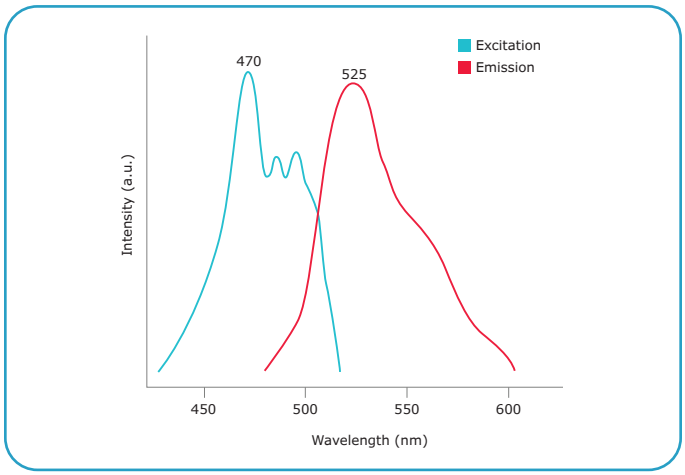


Fig. 5 Schematic of the Excitation and Emission profile of XC. Max excitation and emission wavelengths indicated on each peak. *Note; XC is well suited to the 488nm wavelength of the Argon laser.*

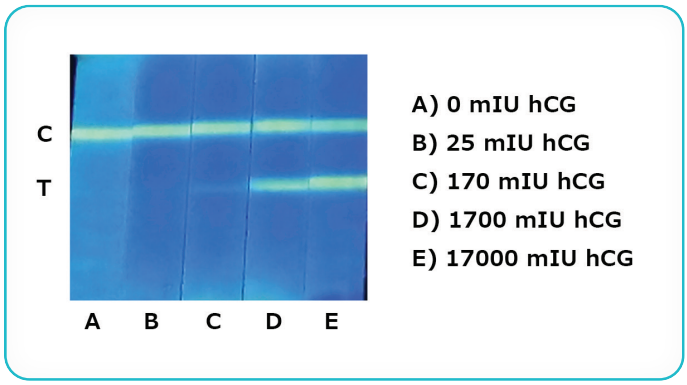


Fig. 4 Qualitative image of Human Chorionic Gonadotropin (hCG) lateral flow test strips utilizing XC fluorescent microspheres viewed under UV light. Key: C= Control line, T=Test line

Table 2: Estapor® XC Fluorescent Microspheres

Product Name	Fluorescence color	Diameter (µm)	Surface COOH (µeg/g)	Cat. No.
F1-XC- 010	Fluorescent green	0.130 -0.190	> 300	39376080
F1-XC- 030	Fluorescent green	0.270 -0.330	200 - 500	39414082
F1-XC- 050	Fluorescent green	0.450 -0.530	1 - 10	80380535

Our New Estapor® Europium Microspheres

We are proud to launch our new Europium microspheres. Labeled with Europium, these microspheres provide several advantages over traditional fluorescent microspheres.

- Significantly improve lateral flow assay sensitivity
- Drastically reduce background fluorescence
- Make assays easier to read and quantify
- Exhibit a much longer Stokes shift than traditional fluorescent labels
- Exhibit an enhanced fluorescent quantum yield facilitating a low detection limit
- Are functionalized with a carboxylated surface for protein conjugation
- Are available in three different size options

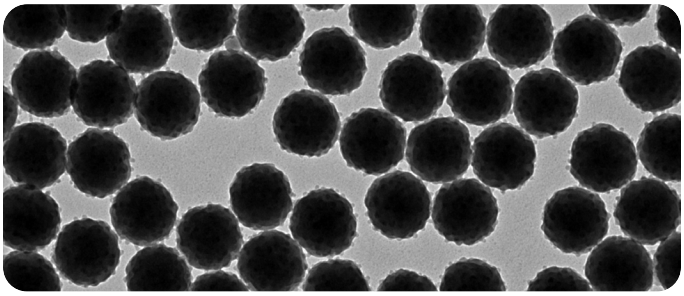


Fig. 6 TEM image of Europium microspheres.

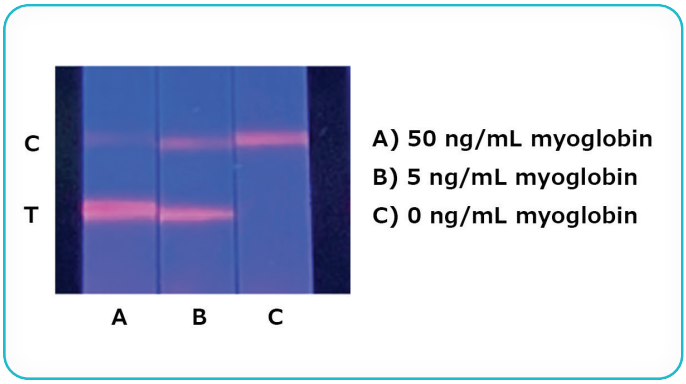


Fig. 7 Myoglobin lateral flow test strips utilizing 300nm Europium microspheres viewed under UV light. Key: C= Control line, T= Test line

Table 3: Estapor® Europium Microspheres

Product Name	Fluorescence color	Diameter (µm)	Surface COOH (µeg/g)	Cat. No.
F1-Eu- 010	Fluorescent red	0.130 -0.190	> 300	80380623
F1-Eu- 030	Fluorescent red	0.270 -0.330	200 - 500	80380624
F1-Eu- 050	Fluorescent red	0.450 -0.530	1 - 10	80380625

Order Europium Microsphere samples today by completing our sample request form:
www.emdmillipore.com/estapor-europium-sample

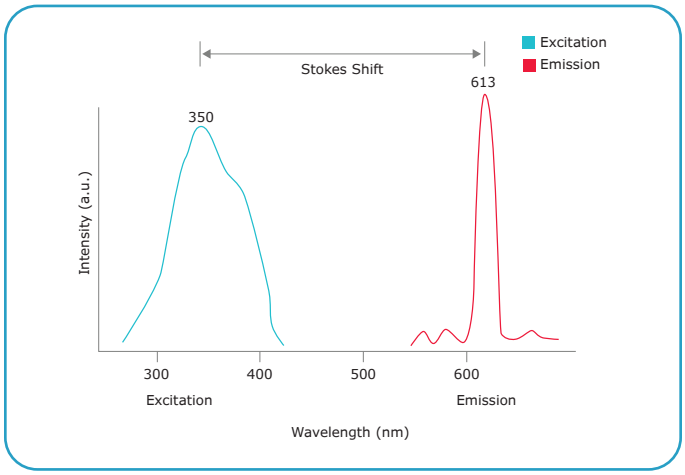


Fig. 8 Schematic of the Excitation and Emission profile of Europium. Max excitation and emission wavelengths indicated on each peak. *Note: the very narrow emission peak, as well as the large Stokes shift of the fluorphor.*

Estapor® Magnetic Microspheres for Quantitative Lateral Flow

There is growing interest in the use of small magnetic microspheres in quantitative lateral flow assays. Their use as magnetic reporter particles and their detection by an external magnetic field applied at the test line constitutes a promising strategy for quantitative high sensitivity lateral flow tests. Our range of small carboxylated superparamagnetic microspheres offer high magnetic susceptibility as well as high surface area making them ideal candidates for use in these types of lateral flow assays.

Table 4: Estapor Magnetic Microspheres for Lateral Flow

Product Name	Diameter (µm)	Ferrite %	COOH Content (µeg/g)	Cat. No.
M1-020/50	0.160-0.240	>50%	130-280	80 380 073
M1-030/40	0.251-0.400	40-60	100-250	80 380 000

Technical Considerations for Lateral Flow Assays

Lateral flow test strips facilitate a user friendly, rapid low-cost analysis for clinical diagnostics. Advancements in lateral flow tests have largely focused on increasing the sensitivity of analyte detection and more recently, the development of quantitative tests often uses organic fluorophores to achieve increased sensitivity. The reliability of Estapor® Microspheres makes them an excellent choice of detection agent, whether your assay is quantitative or qualitative in nature. As a trusted partner in your assay development process, here are a few key considerations to keep in mind as you develop your assays.

Selection of Antibodies

High affinity antibodies should be chosen for the detection of low levels of target analyte.

Note: Even if antibodies paired well in ELISA format, this does not mean they will successfully transfer to lateral flow format. Conducting a pre-screen of multiple antibody pairs in ELISA is recommended.

Selection of Microspheres / Membrane

In lateral flow assays, microspheres are required to move through the porous structure of the lateral flow membrane. The size of the microspheres and membrane pores affect microsphere mobility. Smaller microspheres will move faster than larger microspheres and a more open membrane pore structure will also promote faster movement of microspheres. Thus, microsphere size as well as the membrane pore size should be carefully chosen to maximize assay sensitivity. Larger beads (300 – 500 nm diameter) flow through the membrane at a slower rate, thereby offering greater sensitivity, whilst smaller microspheres (100 – 200 nm diameter) may allow a faster run time but with lower sensitivity.

Compatibility of the Reader

The lateral flow test reader must be compatible with the excitation and emission properties of the fluorescent microspheres. The strips must also have dimensions that are compatible with the scanning system inside the reader.

Viscosity of Sample Liquid

If your target analyte is being analyzed in whole blood, then a filter mechanism for the removal of the red blood cells is critical for the running of the assay. This can be manually performed by the user prior to the test being run. Alternatively, there are filter materials available on the market that can be incorporated into the lateral flow test strip (these materials are typically incorporated within the sample pad of the test strip).

Relative Amounts of Critical Reagents

In developing a new quantitative lateral flow test strip, reagents and materials should be optimized, e.g. antibody concentrations, antigens, sample and buffer formulations will vary. You will need to optimize the antibody load on the microspheres, the concentration of antibody on the test line, the membrane being used, the striping rate (which often depends on membrane pore size) etc. It should be noted that Europium microspheres achieve a higher level of sensitivity in quantitative lateral flow assays compared to standard fluorescent microspheres. Therefore, if you switch from fluorescent microspheres to Europium microspheres, you will likely require fewer Europium microspheres on your test strip.

Quality Assurance You Can Trust

ISO Compliant Manufacturing

Based near Lyon, France our manufacturing site is ISO compliant. Benefiting from over 35 years of microsphere manufacturing experience, our specialized team utilizes state-of-the-art facilities to produce our microspheres to the following standards:

ISO 9001:2015

ISO 14001:2015 (Environmental)

OHSAS 18001:2007 (Health and Safety)

Customer plant audits are available upon request.

Detailed Certificate of Analysis

Each lot of Estapor® Microspheres includes a certificate of analysis providing assurance that:

- The lot complies with all test specifications.
- The lot was produced in compliance with documented manufacturing processed and material specifications.

On each quality certificate, you will find the specific lot information and QC test data for the product.

Custom Products

In most cases, our standard product offering should fulfil your lateral flow requirements. We do however offer custom development services to support demanding applications. Using our state-of-the art facilities, our technical team can provide customized microspheres that will meet your specific needs and requirements. Contact your local account manager to discuss your assay optimization and product customization needs.

Additional Reading

1. Application Note: 'Development of a Quantitative Lateral Flow Test Using Estapor® Europium Microspheres'. emdmillipore.com/estapor
2. Application Note: 'Performance of Estapor® Microspheres and Hi-Flow™ Plus Membranes in a Lateral Flow Assay for Human Chorionic Gonadotropin (hCG)'. emdmillipore.com/estapor
3. Application Note: 'Microsphere Coupling, Two-step EDC/Sulfo NHS Covalent Coupling Procedure for Estapor® Carboxyl-modified Dyed Microspheres'. emdmillipore.com/estapor
4. J.Phys. *Designing novel nano-immunoassays: antibody orientation versus sensitivity.* Appl. Phys. 2010 (43); 474012 (8pp).
5. *Lateral Flow tests and assays: The use of Polymer Microspheres* – In MedLab Issue 1 2010, 15.

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For more information, please visit:
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To discuss your specific product needs,
please contact your local dedicated Account Manager

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