

GENERAL POLICY OF USE



Platform for Biomarkers

- SIMOA HD-X analyzer
- Multiplexing with Luminex Technology
- Standard ELISA service

Version 3
(Jan 2024)

The Achucarro Platform for Biomarkers is equipped since March 2022 with **SIMOA® HD-X analyzer** (Quanterix, Billerica, MA), a fully automated instrument that performs digital, bead-based ELISA immunoassays at unprecedented levels of sensitivity. The instrument offers productivity improvements, greater user flexibility, unparalleled sensitivity, multiplexing and custom assay capability, and best-in-class assay performance across a broad assay menu to empower biomarker research and accelerate drug development (<https://www.quanterix.com/instruments/simoa-hd-x-analyzer/>).

The equipment is property of Achucarro Basque Center for Neuroscience, and was acquired with the support of public competitive founding.

Recently, the Platform for Biomarkers has been expanded by incorporating the standard **ELISA service**, and the ultrasensitive **Multiplex detection service** using **Luminex® xMAP®** (Multi-Analyte Profiling) technology.

This policy has been defined to set the organisation of the correct use of the Platform, and this document is open to improvements and suggestions. Suggestions are taken by the Facility Technician (raffaella.cipriani@achucarro.org).

Platform for Biomarkers reference personnel and contacts

Currently, Estibaliz Capetillo-Zarate (estibaliz.capetillo@ehu.eus) is the researcher in charge of the coordination of the Platform, and Raffaella Cipriani is the researcher responsible for the technical and experimental issues (raffaella.cipriani@achucarro.org).

For any doubt, question, etc, you can contact Estibaliz and Raffaella.

About SIMOA

Our HD-X analyzer is a fully automated instrument for running immunoassays using single molecule array (SIMOA) technology, a digital form of ELISA.

Briefly, the technology improves classical ELISA assays trapping and sealing individual immunocomplexes on paramagnetic beads in thousands of femtoliter sized wells in arrays found on the SIMOA discs. Each well can accommodate only one immunocomplex. At low concentrations, each bead will contain only one bound protein, or none, and single-molecule counting is achieved by counting active (ON) wells (**figure 1**).

By leveraging the digital detection format, this technology enables to precisely measure protein biomarkers with substantially higher sensitivity allowing the application of precision science to precision health.

Main technical specifications

- Throughput of 3 plates every 8-hour shift. About 300 data points per day.
- Workflow, batch (plates or tubes)
- Total assay time is less than 2.5 hours per 96-well plate
- Hands-on time, start-up time <20 minutes
- Sample input, 96-well plate and tubes
- Sample volume, 1 µl * - 100 µl
- Type of samples: CSF, plasma, serum
- Multiplex capability, up to 4-plex
- Custom assay capability
- Assay flexibility, 1, 2 or 3-step assays, with variable incubation time and number of wash steps

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Single Molecule Arrays (SiMoA)

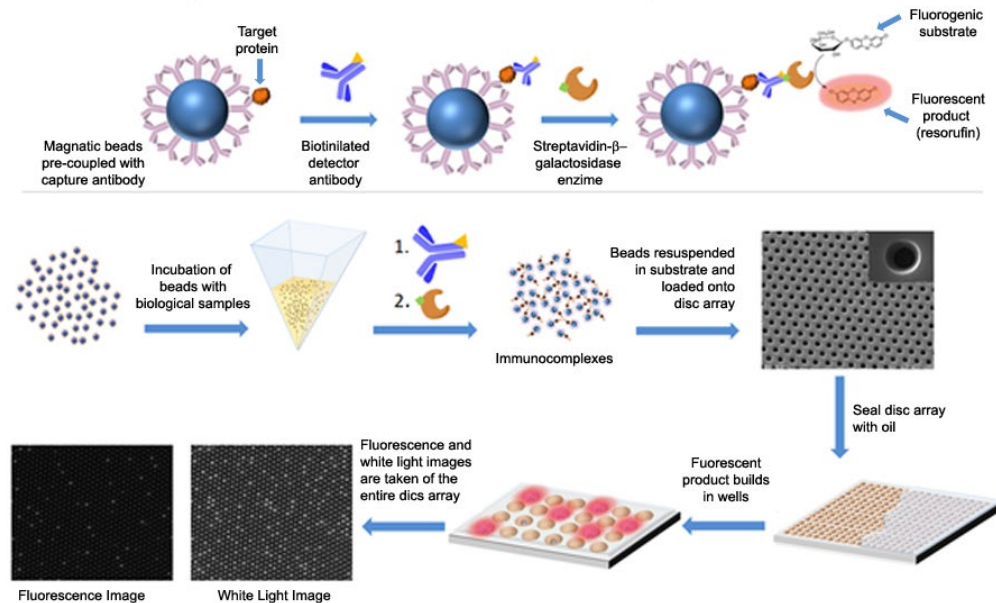


Figure 1. Graphic resume of SIMOA technology. Magnetic beads coated with capture antibody are incubated with a biological sample (**CSF, plasma, serum**). Target proteins bind to the antibody-coupled beads. The beads are then introduced to the biotinylated detection antibody, which binds to the corresponding target protein. In the next step, the enzyme SβG (Streptoavidin-β-Galactosidase) binds to the biotinylated detection antibody, completing the immune complex. Finally, beads are resuspended in a fluorogenic substrate, loaded into microwell arrays, and sealed with oil. Images of the entire disk array are taken and analyzed.

About standard ELISA

ELISA (enzyme-linked immunosorbent assay) is a multi-well plate-based assay technique designed for detecting and quantifying soluble substances such as peptides and proteins. Typically, the antigen is immobilized on a microplate, and then recognized by an antibody that is linked to a reporter enzyme. The incubation of the immunocomplexes with the appropriate substrate for the reporter enzyme produces a measurable product allowing the detection and quantification of the antigen of interest in the sample.

ELISA tests are a rapid and relatively low-cost alternative for the detection and quantification of biomarkers in different type of matrices.

About Multiplex with Luminex® xMAP® technology

Multiplex immunoassays based on Luminex xMAP (Multi-Analyte Profiling) technology are bead-based assays for protein and genes quantification based on the principles of a sandwich ELISA. These assays are available for use with serum, plasma, cell and tissue lysates, cell culture supernatants, and may be suitable for other bodily fluids, and across six species: human, mouse, rat, nonhuman primate, porcine, and canine.

Preconfigured and custom panels are available.

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Figure 2 shows a resume of the xMAP technology, that allows to measure up to 80 different protein analytes simultaneously per well. Capture antibodies are bound to Luminex beads which are internally dyed. The conjugation of a specific antibody to a distinct bead allows for analysis of multiple analytes in a single well, e.g. IL-9 is captured via bead region #12 (A). Samples are mixed with the bead sets. Analytes of interest within the sample are bound by the capture antibodies. Fluorescently labelled (Phycoerythrin) detection antibodies specific to the analytes of interest are added, forming an antibody-antigen sandwich (B). Completed assays are read on a Luminex instrument where one laser classifies the bead type to determine the analyte that is being detected while a second laser determines the magnitude of the bound analyte (PE-derived signal) (C).

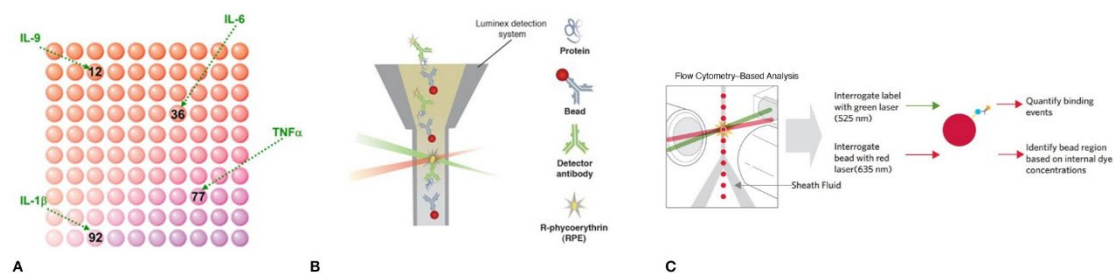


Figure 2. Graphic resume of LUMINEX technology for protein analytes detection (adapted from ThermoFisher Scientific).

Basic working conditions and procedures

- At Achucarro, we are offering two options to researchers interested in using **SiMoA HD-X** analyzer.

1) In collaboration

If it is established that the use will be in collaboration, the interested researcher will bear the cost of the tests, reagents, buffers and consumables necessary to run the desired number of samples. The researcher will place the order with its billing address and the order will be sent to Achucarro where the samples will be run. Being in collaboration, there are no other added costs. Collaboration will be considered when both Achucarro and external researchers agree.

If you consider the collaboration option, we would appreciate it if you could send us a short summary of the work for our consideration.

2) As a service

In the event that the quantification of the samples is as a service, the interested researcher will bear with the cost of the assay/s, and with the cost of the service that depends of the number of samples and the number of different assays. The cost of the service also includes reagents, buffers and consumables necessary to run the desired number of samples. 21% VAT will be added to the total cost of the service (**table 1**).

- Regarding the **ELISA and Multiplexing services**, the interested users will bear with the cost of the assay/s, and with the cost of the service that is in price per working hours (**table 1**).

The Multiplex service includes the analysis of the plate/s with the instrument Luminex 200 located in BioBizkaia, with the additional cost reported in **table 2**.

21% VAT will be added to the total cost of the service.

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Table 1. Price of the SIMOA, Multiplex, and standard ELISA services.

		Internal	External (Public Research Organization)	Private	discount
SIMOA HD-X ANALYZER (price per sample)*	up to 38 samples	18.39 €	22.99 €	41.38 €	0
	39 - 76 samples	17.47 €	21.84 € + 21% VAT	39.31 € + 21% VAT	5
	77 - 114 samples	15.72 €	19.66 €	35.38 €	10
	from 115 samples	13.37 €	16.71 €	30.07 €	15
Multiplex with Luminex 200 (price per hour)		12.38 €	15.48 € + 21% VAT	-	
ELISA (price per hour)		8.26 €	10.32 € + 21% VAT	-	

* in the case of more than one assay, samples must be multiplied per n° of assay
 es. 38 samples, 2 different assays: TOTAL NUMBER OF SAMPLES IS (38*3) = 76
 es. 38 samples, 3 different assays: TOTAL NUMBER OF SAMPLES IS (38*2) = 114

Table 2. Price for the use of Luminex 200 instrument (BioBizkaia)

		Internal	External
		Price per plate	Price per plate
Luminex 200 (Biobizkaia)	1 plate/day	135.00 € + 21% VAT	180.00 € + 21% VAT
	2 plates or more/day	69.00 €	92.00 € + 21% VAT

Contact us (estibaliz.capetillo@ehu.eus, raffaella.cipriani@achucarro.org) to provide you a quote.

User Priorities (according to affiliation):

1. **Achucarro and UPV/EHU** users have the maximum priority on the use of the equipments and services offered by the Platform for Biomarkers; after them,
2. Other non-for-profit research or technological centres within the **Basque Science Community** (or abroad); and finally,
3. Companies and private entities.

In exceptional cases, if a user of this third group has a special-time related need and according to their pricing policy, they could be given priority over other users.

Acknowledgement

Users MUST mention the use of this platform in their publications and research outcomes as:

Achucarro Basque Center for Neuroscience – Platform for Biomarkers (Leioa, Spain)