GENERAL POLICY OF USE

Platform for Biomarkers

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

Version 4
(March 2025)

The Achucarro Platform for Biomarkers was created in 2018 with the **Simoa® HD-1 analyzer** (Quanterix, Billerica, MA). In March 2022, the platform was upgraded with the **Simoa® HD-X analyzer,** a fully automated instrument performing digital, bead-based ELISA immunoassays at unprecedented levels of sensitivity (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 (raffaela.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 Raffaela Cipriani is the researcher responsible for the technical and experimental issues (raffaela.cipriani@achucarro.org).
For any doubt, question, etc, you can contact Estibaliz and Raffaela by e-mail or by phone (**+34 946018309**).

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 kits, reagents, buffers and consumables necessary to run the desired number of samples. The researcher will place the order to Quanterix 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 an additional cost per plate/day (more information upon request).

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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)[1, 2]	up to 37 samples	19.00 €	23.75 € + 21% VAT	42.75 € + 21% VAT	*0*
	38 - 74 samples	18.05 €	22.56 €	40.61 €	*5 %*
	75 - 111 samples	17.10 €	21.37 €	38.47 €	*10 %*
	from 112 samples	16.15 €	20.19 €	36.33 €	*15 %*
[1] in the case of more than one assay, samples must be multiplied per nº of assay to obtain the total number of samples es. 37 samples, 2 different assays: TOTAL NUMBER OF SAMPLES IS (37*2) = 74 es. 37 samples, 3 different assays: TOTAL NUMBER OF SAMPLES IS (37*3) = 111 [2] Cost of the kit not included.					
Multiplex with Luminex 200 (price per hour)		12.80 €	15.99 € + 21% VAT	28.78 € + 21% VAT	
ELISA (price per hour)		8.55 €	10.66 € + 21% VAT	19.19 € + 21% VAT	

Contact us (estibaliz.capetillo@ehu.eus, raffaela.cipriani@achucarro.org) for a free 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)

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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 Simoa technology improves classical ELISA assays trapping and sealing individual bead-immunocomplexes in femtoliter-sized wells organized in arrays on the Simoa discs. The compartmentalization of each bead-immunocomplex allows for enzyme bound to the capture antibody to produce sufficient fluorescence in each well to be detectable, even when just a single molecule is present, such as at very low concentrations. As a result, the analyzer produces a map of the array (each array contains more than 200,000 wells) where each well can be digitally analyzed as either “on” (containing a target molecule) or “off” (**figure 1**). The proprietary Simoa algorithm then converts the measurement into a concentration which reaches femtomolar levels for most analytes.

This revolutionary approach to digitalizing ELISA offers up to **1000-fold improvement in sensitivity** over current immunoassay techniques (source: Quanterix website).

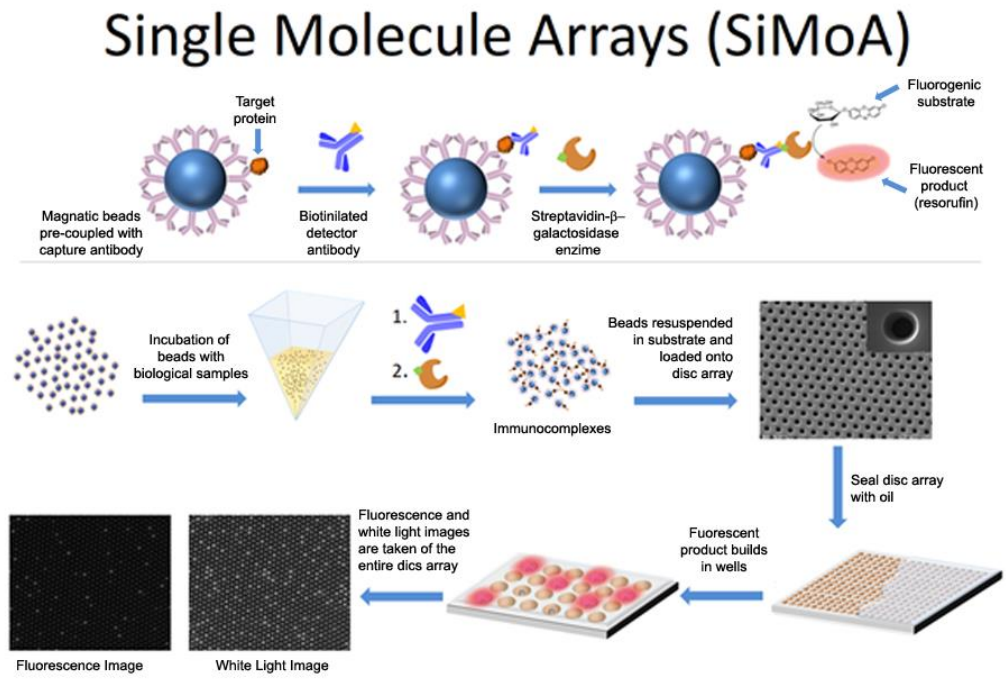

Figure 1. Graphic resume of Simoa technology. Magnetic beads coated with capture antibody are incubated with a biological sample (**CSF, plasma, serum**). Target proteins bind to the antibody-coupled beads. The beads are then introduced to the biotinylated detection antibody, which binds to the corresponding 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.

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Main technical specifications of Simoa HD-X analyser:

- Sensitivity: up to 1000x greater sensitivity than current immunoassays
- Dynamic Range: >4 logs
- Precision: <10% CVs
- Complete automation
- Built-in touch-screen control and comprehensive data analysis tools
- Throughput of 3 plates every 8-hour shift. About 300 data points per day.
- Workflow, batch (plates or tubes)
- Total assay time: < 2.5 hours per 96-well plate
- Run set up time: <20 minutes
- Sample input: 96-well plate and tubes
- Sample volume, 1 µl * - 100 µl (*<10 µl requires pre-dilution step)
- Type of samples: CSF, plasma, serum
- Multiplex capability, up to 6-plex
- Custom assay capability

Here is a list of some of the singleplex and multiplex kits commercially available at Quanterix (last update: 2025):

SIMOA BEAD-BASED ADVANTAGE ASSAYS - January 2025

Singleplex Assay/Analyte (96 tests)	LOD (pg/mL)	Analytical LOQ (pg/mL)	Sample type †	Sample Volume (µL) ††
ALZPath p-tau 217 Advantage PLUS	0.0008	0.00326	E	33
BD-Tau Advantage PLUS	0.044	0.133	E, S, C	25 (E & S); 1 (C)
BDNF	0.0042	0.0293	E, S, C	0.5 (E & S); 50 (C)
C-Peptide	0.013	0.021	E, S	1 - 25.5
GFAP Advantage PLUS	0.138	0.635	E, C	25 (E); 0.25 (C)
GM-CSF	0.0019	0.0103	E, S	25
HIV p24	0.0027	0.01	E, S	154
IFNa Multi Subtype Advantage PLUS	0.002	0.018	E, S	50
IFNγ	0.015	0.098	E, S	50
IL-1β Advantage PLUS	0.002	0.008	E, S	25
IL-4	0.0046	0.039	E, S	50
IL-5 Advantage PLUS	0.002	0.01	E, S	25
IL-6 Advantage PLUS	0.01	0.028	E, S	25
IL-7	0.009	0.103	E, S	25
IL-10 Advantage PLUS	0.015	0.048	E, S	25
IL-12p70	0.005	0.017	E, S	25
IL-15	0.003	0.0062	E, S	25
IL-17A Advantage PLUS	0.002	0.012	E, S	25
IL-22 Advantage PLUS	0.008	0.061	E, S	25
IP-10	0.052	0.177	E, S	25
NF-Light Advantage PLUS	0.062	1.24	E, S, C	26 (E & S); 0.25 (C)
PD-L1	0.055	0.617	E, S	5
PlGF	0.064	0.3	E, S	30
PSD95 Advantage PLUS	0.173	0.823	E, S, C	25
p-Tau 181 Advantage PLUS	0.724	7.27	E, S, C	25 (E & S); 10 (C)
p-Tau 217 Advantage PLUS	0.001	0.01	E, S	86
p-Tau 231 Advantage PLUS	0.232	1.23	E, C	50 (E & S); 25 (C)
SARS-CoV-2 N Protein Antigen	0.099	0.313	NP Swab	25

SARS-CoV-2 Spike IgG	15	8,200	E, S	0.1
SNAP-25	0.88	2.56	C	25
SNAP-25 v2	1.47	2.56	C	25
sTREM2 Advantage PLUS	0.9	66	E, C	6.25
Tau	0.019	0.061	E, S, C	38 (E & S); 15.2 (C)
TDP-43	2.480	8.230	E, S, C	25
TGFβ	0.137	0.514	E, S	8.5
TNFα	0.016	0.034	E, S	25

Multiplex Assay (96 tests)	Analytes	LOD (pg/mL)	Analytical LOQ (pg/mL)	Sample type	Sample Volume (µL)
Cytokine 4-Plex A (C4PA)	IL-1β	0.002	0.01	E, S	25
	IL-6	0.008	0.028		
	IL-10	0.033	0.143		
	TNFa	0.061	0.481		
Cytokine 4-Plex B (C4PB)	IL-4	0.003	0.01	E, S	25
	IL-5	0.004	0.01		
	IL-13	0.004	0.034		
	IL-17A	0.002	0.008		
Cytokine 4-Plex C (C4PC)	IL-2	0.005	0.019	E, S	25
	IL-6	0.008	0.031		
	IL-8	0.014	0.07		
	IFNγ	0.012	0.075		
Neurololgy 2-Plex A (N2PA)	Aβ40	0.262	0.353	E, C	25 (E); 0.25 (C)
	Aβ42	0.111	0.24		
Neurololgy 2-Plex B (N2PB)	NF-light	0.08	0.402	E, C	25 (E); 0.25 (C)
	GFAP	0.111	0.635		
Neurololgy 4-Plex E (N4PE)	Aβ40	0.133	0.353	E, S, C	25 (E); 0.25 (C)
	Aβ42	0.13	0.239		
	GFAP	0.139	0.402		
	NF-light	0.072	0.635		
Neurololgy 4-Plex D (N4PD)	GFAP	0.121	0.805	E, S, C	25 (E & S); 10 (C)
	NF-light	0.094	0.36		
	BD- Tau	0.029	0.259		
	UCH-L1	0.577	3.48		

† C = CSF, E = EDTA plasma, S = serum

† † Volumes shown are for at bench dilutions. On-instrument dilutions require higher sample volumes.

You can find more information about Quanterix ready-to-use Simoa® kits at the following link: https://www.quanterix.com/simoa-assay-kits/.

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 using Luminex® xMAP® (Multi-Analyte Profiling) technology are bead-based assays for **proteins and RNA 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 species: human, mouse, rat, nonhuman primate, porcine, and canine. **Figure 2** shows a resume of the xMAP technology.

Preconfigured and custom panels are available, allowing the simultaneous detection and quantitation of up to 80 secreted proteins (including over 600 cytokine, chemokine, growth factors, and other protein targets) in a single 96-well plate format.

Furthermore, this multiplex technology can be used for gene expression analysis, as the combination with branched DNA (bDNA) technology enables the measurement of up to 80 gene targets.

Here is a list of **useful links**:

- https://us.diasorin.com/en/luminex-ltg/xmap-technology#videos
- https://www.thermofisher.com/es/es/home/life-science/antibodies/immunoassays/procartaplex-assays-luminex/procartaplex-immunoassays.html
- https://www.thermofisher.com/es/es/home/life-science/antibodies/immunoassays/procartaplex-assays-luminex/quantigene-plex-assays.html
- https://www.sigmaaldrich.com/ES/es/configurators/milliplex

More information about covered targets and combinations upon request.

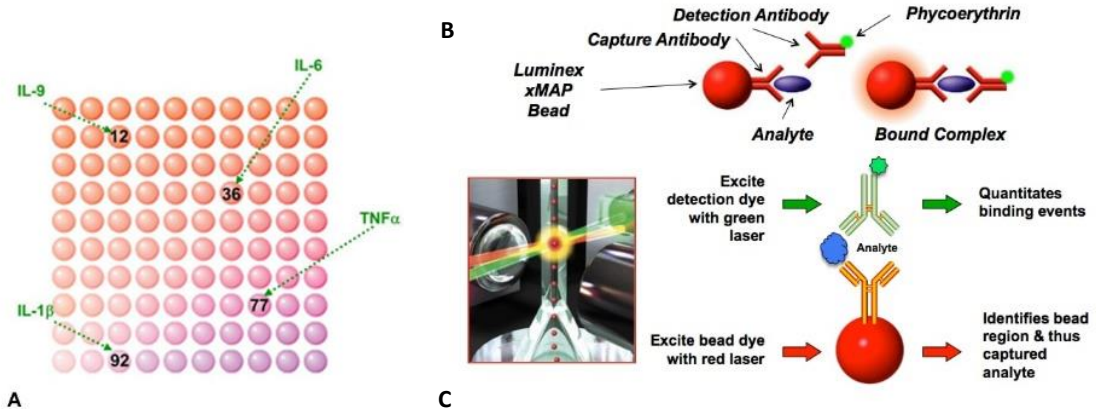

Figure 2. Graphic resume of LUMINEX xMAP technology for protein analytes detection (adapted from ThermoFisher Scientific). Briefly, capture antibodies are bound to Luminex beads which are internally dyed. The conjugation of a specific antibody to a distinct bead allows for analysis of multiple analytes 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**).