

## Overcoming the Cost and Performance Limitations of ELISA with xMAP® Technology

### Introduction

For nearly half a century, immunoassays have been the primary source for detection of analytes of interest in biological samples for both life science research and clinical diagnostics. This began with the quest to measure insulin levels and culminated in the development of the radioimmunoassay (RIA) by Yalow and Berson (1960). The desire to use less hazardous detection methods than radioisotopes led to the development of the enzyme-linked immunosorbent assay (ELISA) by Engvall and Perlmann (1971). The advantages of ELISA are its ease-of-use, flexibility, the ability of any lab to build assays, and low cost as evidenced by nearly 10,000 studies published per year utilizing the technique (Lequin, 2005).

While widely utilized, ELISA has limitations. An ELISA is typically performed in a 96-well microplate and the wells are coated with capture antibody. In order to capture antigen of interest from the sample, a relatively large amount of sample is required. The large surface area of the individual microplate wells and the hydrophobic binding of capture antibody can lead to non-specific binding and increased background. Also, most ELISAs rely upon enzyme-mediated amplification of signal in order to achieve reasonable sensitivity.

### xMAP Technology as an Alternative to ELISA

In the past 15 years, a new technology has emerged that offers the benefits of the ELISA, but also enables the added value of higher throughput, increased flexibility, reduced sample volume, and low cost with the same workflow as ELISA. Luminex's® xMAP Technology has been utilized in over 7,700 publications ([www.luminexcorp.com/bibliography](http://www.luminexcorp.com/bibliography)), and can be applied to both protein and nucleic acid applications. The suspended beads allow for assay flexibility in a single-plex or multiplex format. Since the beads have the capture antibody immobilized on their much smaller surface area compared to a microplate well, smaller sample volumes are required and non-specific binding is reduced (Carson & Vignali 2000). Smaller samples are important when working with limited sample types such

as cerebrospinal fluid, synovial fluid, tears, mouse serum, etc. Recent improvements by Luminex include the new MAGPIX® system and the introduction of magnetic MagPlex® microspheres which have reduced the cost of performing a single-plex assay as expensive filter plates are no longer required.

The Thomas Joos laboratory at Natural and Medical Sciences Institute (NMI) at the University of Tuebingen performed a comparison of a sandwich immunoassay on ELISA and xMAP Technology using the MAGPIX system from Luminex. The assay was built to detect the soluble form of the Tumor Necrosis Factor Receptor 2 (TNF RII). Parallel assays were built using the same antibodies and recombinant standard protein. The same material preparations (e.g. serial dilutions of standard proteins) were utilized to achieve the best possible comparison.

### Reduce Costs and Total Labor Time using xMAP Technology

The costs for the bead-based sandwich immunoassay and ELISA were calculated based on experiments performed at NMI. The calculations are based on the exact requirements for a 96-well plate. Actual costs could be slightly higher due to materials such as standards that could be used for a large number of assays, but might have to be disposed of due to shelf-life or thawed aliquots that have to be discarded due to shelf life issues. Both costs were calculated using a seven-point standard curve and assuming that there are 80-samples per plate and 16 wells used for background and standards. The costs were based in Euros and the MAGPIX assay was less than half the cost of the standard ELISA, utilizing the same pair of antibodies and the same recombinant standard.

† In addition to the numerous customer developed assays on xMAP Technology, Luminex has partnered with over 50 industry leading companies to develop commercialized assays for immediate use in both the life science research and clinical diagnostic markets. ([www.luminexcorp.com/partners](http://www.luminexcorp.com/partners)).

**Figure 1. Cost comparison of xMAP assay on MAGPIX versus an ELISA assay.**

### MAGPIX\*

Item	Price	Price/Plate (96 wells)
ELISA microplate	0.97 € / plate	0.97 €
MagPlex Beads	\$630 / mL †	11.20 € †
Capture Antibody	330.00 € / 500 µg	0.70 €
Standard Protein	345.00 € / 50 µg	0.12 €
Detection Antibody	425.00 € / 50 µg	7.40 €
Goat Serum	49.00 € / 25 mL	0.12 €
Strep-PE	250.00 € / 1 mL	1.75 €
Microplate Assay Sealing Film	49.00 € / 100 pieces	0.49 €
Buffers, etc...		1.00 €
	TOTAL	23.75 €

\*All costs reported in Euros (€).

### ELISA\*

Item	Price	Price/Plate (96 wells)
ELISA microplate	0.97 € / plate	0.97 €
Capture Antibody	330.00 € / 500 µg	12.67 €
Standard Protein	345.00 € / 50 µg	0.12 €
Detection Antibody	425.00 € / 50 µg	24.48 €
Goat Serum	49.00 € / 25 mL	0.38 €
Streptavidin-HRP	250.00 € / 1 mL	12.00 €
TMB-Substrate	49.00 € / 100 mL	4.70 €
Microplate Assay Sealing Film	49.00 € / 100 pieces	0.49 €
Stop solution (1M HCl)	13.55 € / 1L	0.19 €
	TOTAL	56.00 €

In addition to the assay cost comparison, the investment in capital equipment required to run these assays should be considered. With the recent launch of the new MAGPIX system, access to xMAP Technology and the benefits of multiplexing has now become more affordable†. At the cost of a high performance ELISA plate reader, researchers can now obtain more information from less sample volume using a similar workflow to ELISA. The total-assay-time (TAT) and hands-on-time (HOT) were measured for both assay formats. Time for couple detection antibodies

to beads and coating of ELISA plates was not added, as this is done for many assays at once. The TAT is similar for both assay formats, although the ELISA has more steps. This is because the MAGPIX has a longer read time than an absorbance plate reader. Labor costs will be similar for the two assays when comparing a single-plex assay. If multiple analytes are being examined, then labor costs would increase for the ELISA, but remain the same for MAGPIX. Magnetic beads allow for automation of workflow and better reproducibility compared to the previous generation of polystyrene beads.

**Figure 2. Time comparison of ELISA and xMAP assays**

### MAGPIX

	Assay Time min	Hands-on Time min
Blocking of (ELISA) microtiter plate	30	5
Dilution of standard protein and preparation of samples ( 1 standard curve in duplicate and 80 samples)	40	40
Pipetting of 100 µL sample or standard to each well	10	10
Incubation	120	
Washing step (3 x 100 µL) using magnetic plate	5	5
Preparation of detection antibody solution	2	2
+ 30 µL of the detection antibody solution per well	3	3
Incubation with detection antibody	60	
Washing step (3 x 100 µL) using magnetic plate	5	5
Preparation of streptavidin-phycoerythrin solution	2	2
+ 30 µL Strep-PE per well	3	3
Incubation	30	
Washing step ( 3 x 100 µL) using magnetic plate	5	5
Assay read-out	80	5
<b>Total assay time</b>	<b>6 h 30 min</b>	
<b>Total Hands-on time</b>		<b>1 h 35 min</b>

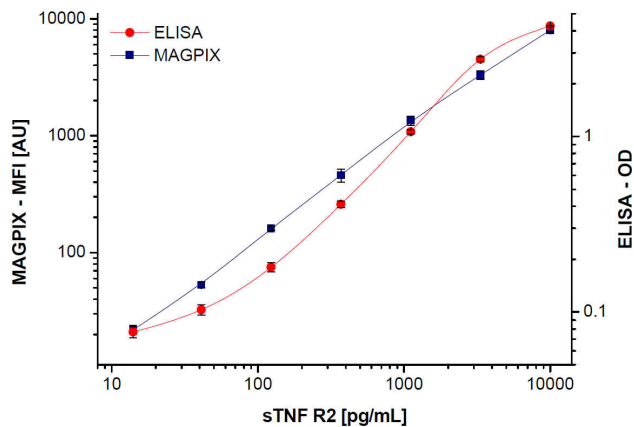
### ELISA

	Assay Time min	Hands-on Time min
Dilution of standard protein and preparation of samples ( 1 standard curve in duplicate and 80 samples)	40	40
Pipetting of 100 µL sample or standard to each well	10	10
Mixing	1	1
Incubation	120	
Washing step (3 x 400 µL)	5	5
Preparation of detection antibody solution	2	2
+ 100 µL of the detection antibody solution per well	3	3
Incubation at room temperature	120	
Washing step (3 x 400 µL)	5	5
Preparation of streptavidin-HRP solution	2	2
+ 100 µL streptavidin-HRP per well	3	3
Incubation	20	
Washing step ( 3x 400 µL)	5	5
Dilution of substrate solution	1	1
+ 100 µL substrat solution per well	2	2
Incubation	30	
+ 50 µL stop solution per well	2	2
Assay read-out	10	5
<b>Total assay time</b>	<b>6 h 20 min</b>	
<b>Total Hands-on time</b>		<b>1 h 40 min</b>

## Analytical Performance of xMAP Technology is Comparable to ELISA

The range of both assays was determined using a three-fold serial dilution of the standard recombinant protein, starting at 10,000 pg/ml with a total of seven standard concentrations. Both assays performed over the range of 14 to 10,000 pg/ml, with the MAGPIX assay having a more linear range than the ELISA (Figure 3).

**Figure 3. Standard curves of ELISA and xMAP assay performed on MAGPIX.**



The limit of detection (LOD) of the TNF RII MAGPIX assay was 4 pg/ml and the limit of quantitation (LOQ) was 10 pg/ml. For the ELISA, the LOD was 9 pg/ml and the LOQ was calculated to be 50 pg/ml. LOD was defined as the recalculated concentration obtained by adding three standard deviations to the background reading. LOQ was calculated using the same process, but with the addition of ten standard deviations to background. The MAGPIX assay had a two-fold more sensitive LOD and was five-fold more sensitive for LOQ. The LOD for each assay is going to be dependent upon the proper selection of antibody pairs and standards. Assays can reach detection limits of less than 1 pg/ml.

Analytical comparison of the two assay formats demonstrates that the bead-based xMAP assay can be used to obtain comparable results with improved efficiency and performance over the traditional ELISA format. Historical data obtained using ELISA can be maintained while transitioning to xMAP assays on the MAGPIX system, enabling researchers to continue long-standing studies and access the added benefits of xMAP Technology. In addition to the benefits demonstrated by a single-plex assay

format, conversion to xMAP Technology provides researchers the added benefit of multiplexing several tests in the same well; further increasing efficiency and reducing time and costs in the laboratory.

## Publications Support Findings in a Multiplex Format

In addition to the recent study outlined above, there have been numerous laboratories that have evaluated the performance of xMAP Technology over the years (de Jager, et al. 2003; de Jager et al. 2005; du Pont et al. 2005; Codorean et al. 2010; Richens et al. 2010). Coincident with the NMI study a group at Bristol-Myers Squibb (BMS) was pursuing development of a more sensitive assay for IL-23 than they could obtain commercially† (Rizzi et al. 2010). They utilized the same pair of antibodies as available in one of the two commercial ELISAs they evaluated. The Luminex assay provided the best precision and accuracy, had the widest range of detection, and the highest sensitivity compared to the two commercial kits. Rizzi et al. also combined the single-plex IL-23 assay with other biomarkers and found that it performed well in either a single-plex or multiplex format. In the single-plex format the xMAP assay had excellent correlation to the two ELISAs ( $r^2 \sim 0.9$ ). In the past only multiplex studies had been performed comparing xMAP to ELISA. The BMS group provided enough information in the paper to do a cost analysis similar to the NMI group. The cost of the xMAP assay was less than one-fifth of the least expensive commercial ELISA they used and less than one-sixteenth of the more expensive commercial kit.

The explanation of why the xMAP-based assays perform better and are less expensive than ELISA is based on several factors. The first is that the xMAP assays are based on direct fluorescence detection as opposed to colorimetric detection mediated by an enzyme, resulting in better sensitivity. Second, capture antibodies have higher avidity and lower background due to covalent coupling to beads as opposed to passive coating of the ELISA plates. This leads to a higher density of capture antibody per surface area and the capture antibodies will not wash off during the assay. The higher background of ELISA plates is due to the fact that they have a much larger surface area than the combined area of 2,500 beads, which is more prone to non-specific binding of detection antibody (Carson & Vignali 2000). If blocking of the large surface area of the microplate is not performed correctly, any proteins can bind. Finally, cost is reduced because there is less capture antibody used due to the smaller surface area.

† The manufacturer's suggested sales price of the MAGPIX is \$35,000. Please contact Luminex or a Luminex Partner authorized to distribute the MAGPIX system for pricing and information.

## Conclusion

xMAP Technology offers all the benefits of the ELISA with the added value of higher throughput, increased flexibility, reduced sample and lower costs with the same workflow as ELISA. Both Rizzi et al. and NMI demonstrated that Luminex assays using the same antibody pairs out-perform ELISA and show significant cost savings based on the cost of materials, notably antibodies, used in the assay. Labor should be equal in a single-plex comparison as the assay protocols are approximately the same length. However, Luminex offers the flexibility to multiplex as well, and in that case labor cost would decrease by a factor of the multiplex level, i.e. labor costs would decrease by one-half if a two-plex was performed.

The MAGPIX system using xMAP Technology offers significant advantages over ELISA, especially material savings which can be very substantial, when compared to commercial ELISA kits. Researchers do not have to multiplex to save money by moving to the xMAP format. There is also the option of multiplexing which can save time and sample proportional to the number of analytes.

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