

# 3

## 3.8 Multiplex Assay Detection

267 Luminex®

270 Full Moon Antibody Arrays



# Luminex®

## xMAP® Multiplexing assay development & testing services

The popularity multiplex assay platform, such as the xMAP technology from Luminex Corporation, is quickly rising, replacing traditional assays such as ELISA and MicroArray technologies for measuring the reaction of a target compound or molecule to the influence of outside parameters.

Choosing Eurogentec as your partner for the development of your Luminex® assay is the guarantee to benefit from:

- A unique technology combining multiplexing, quantification and high-throughput
- A way to overcome ELISA limitations
- A gain of time and money
- The experience and ability of a dedicated team

## Technology

Multiplex assay platforms involve microspheres in the range of 3 to 6 microns that are internally dyed with fluorescent materials (Figure 1) and then coated with a substrate unique to the desired test to be performed. These microspheres, alone or in combination with other coated microspheres, are then suspended in the sample, typically in the wells of a filter bottom microplate. When the biological samples are assayed, the target molecules bind to the coated microspheres. Two lasers excite the microsphere's internal dyes that identify each microsphere particle (Red laser), and reporter dye captured during the assay (Green laser) (Figure 2). The resulting fluorescence can then be detected via flow cytometry.

## Benefits

The motivation behind the shift to multiplexing assay is the ability to:

- Simultaneously analyze multiple targets within one reaction/sample
- Quantification of the target molecule
- High level of throughput. Substantial cost savings and convenience

## Features

The chief advantages of a multiplex assay platform are an increase in the amount, sensitivity and precision of information collected, as well as a decrease in costs associated with cell culture, enzymes/reagents, compounds, labels and other consumables.

The fluorescent-bead-based detection assay has a clear advantage above the conventional ELISA:

- Ability to detect large numbers of analytes simultaneously.
- Due to this liquid phase, the chance of interaction of proteins with their complementary antibodies will increase. This effect will lead to less non-specific binding and thus to lower background signals.
- The volume required to detect all analytes would be sufficient to test a single cytokine by ELISA.
- This multiplex assay is sensitive and accurate since each fluorescence signal is the mean of 100 measurements of a single microsphere, and each microsphere represents an assay by itself.

- Each standard curve can be adjusted to a biological range in which a sample can be expected depending on the origin of the specimen. With this wide dynamic range of standard curves, samples do not have to be diluted or concentrated.
- The xMAP technique can be used to generate quantitative and comparative analyte data in a given time period which is shorter than that required by a standard ELISA procedure.

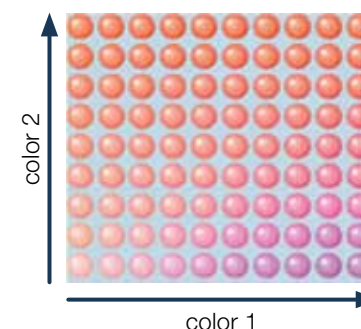


Figure 1: Using a two-dye method, Luminex® produces 100 distinct beads sets.

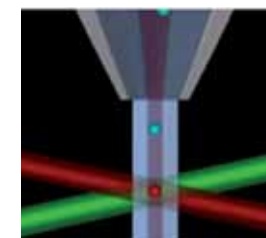


Figure 2: Two lasers excite the microsphere's internal dyes that identify each microsphere particle (Red laser), and reporter dye captured during the assay (Green laser).

## Applications

In principle any soluble factor can be measured with xMAP technology including:

- ▣ Subclasses of immunoglobulins
- ▣ Virus-specific antibodies
- ▣ Pathogen capsular polysaccharides analysis
- ▣ Single-nucleotide polymorphisms
- ▣ Gene expression

The microspheres can be coated with multiple molecules, like antibodies or nucleic acid fragments allowing the design of the perfect multiplex assay dedicated to your needs.

- ▣ Each microsphere set is coated with a capture reagent (e.g. antibody or oligonucleotide).
- ▣ The sample analytes are specifically captured.
- ▣ The detection is made using a fluorescent dye.
- ▣ Interrogations are made by a **Red** laser to identify the bead type and by a **Green** laser to determine the amount of bound analyte.

## Our Services

Eurogentec supplied their customers with immunological reagents: Antibody, Peptides, gene expression service, ELISA-based assays ...

As one stop shop company, now we can put all these products to work more efficiently through our Custom Assay Development. Eurogentec and Luminex® joined forces to provide industry and academia scientists with a unique service platform dedicated to the design, development and testing of multiplex assays you always have been waiting for.

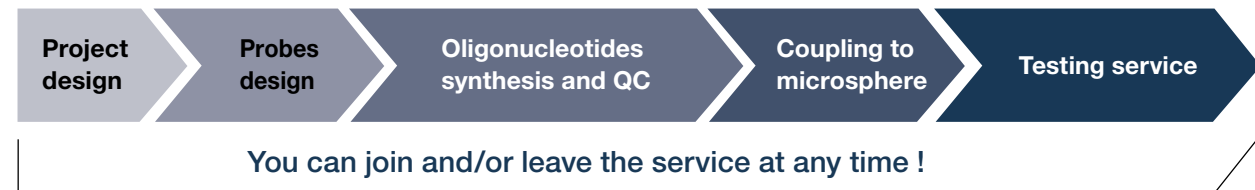
## Immuno-Assay Development

Eurogentec can guide you through all the steps from your needs to your multiplex immunoassay.

### Custom Gene Expression



Eurogentec can provide you with quantitative measurements of multiple RNA targets simultaneously with unparalleled accuracy and precision. A wide variety of starting material is compatible: cultured cells, whole blood, or either fresh, frozen or formalin-fixed, paraffin-embedded tissue.



## Testing Services

If you already have your own assay, either a commercial kit or a homemade protocol, Eurogentec can provide you with a full testing service, avoiding any investment into additional equipment. Choosing Eurogentec as your partner for the development of your Luminex assay is the guarantee to benefit from:

- ▣ The technology combining multiplexing, quantification and high throughput
- ▣ A way to overcome ELISA limitations
- ▣ A gain of time and money
- ▣ The experience and ability of a dedicated team

## Contact

Before going into technical steps, your project will be fully analysed in order to evaluate its feasibility and to build the strategy, providing the highest chance of success.

You will be provided with a detailed quotation, mentioning all steps and associated costs in order to facilitate your decision process. Please contact us to meet our specialists and start designing the multiplex assay made for you: [luminex-info@eurogentec.com](mailto:luminex-info@eurogentec.com)

## Luminex® Epitope Mapping Service

1. Peptides are designed all along the antigenic sequence. Generally 10 amino acid peptides are generated with 5 amino acid overlapping.

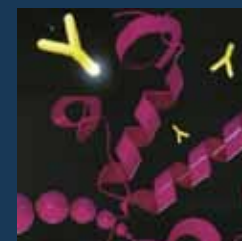
```

▣ KNC SHIQPWE
▣      IQPWETDCLS
▣          TDCLSCLPER
▣              CLPERQDEYD
▣                  QDEYDPKGPk
  
```

2. Peptides are synthesized and biotinylated.
3. Biotinylated peptides are coupled to avidine coated Luminex® beads (LumAvidin beads). Each peptide is coupled to thousands of beads and each bead presents many copies of the same peptide.
4. Remaining free avidin sites are saturated with free biotin.
5. Thousands beads coupled to up to 20 different peptides are dispensed into a well.
6. Various dilutions of your antibody or serum are incubated with the coupled beads.
7. A secondary antibody coupled to phycoerythrin and specifically directed to the primary antibody is added into the well.
8. For each peptide, about a hundred microbeads are analysed and identified by the Luminex® red laser while the phycoerythrin signal is recorded by the green laser.

For a single peptide you obtained a large amount of reliable and reproducible data (min. 100). Moreover positive and negative controls are carried out (same controls as explained in the starting QC part).

## Mouse monoclonal antibody development



Benefit from our vast experience, and continual development in monoclonal production

- ▣ See chapter 3.2

# Full Moon Antibody Arrays

## Pathway & Phosphorylation Antibody MicroArrays

Antibody MicroArray provides a high-throughput platform for efficient and accurate profiling of protein expressions. Our Pathway Focused Antibody MicroArrays and Phosphorylation Antibody MicroArrays are designed for researchers to study highly relevant proteins in their specific research fields. The antibodies are covalently immobilized on high quality glass surface coated with a proprietary 3-D polymer materials to ensure high binding efficiency and specificity. Arrays utilize fluorescent detection and can be scanned on all MicroArray scanners that are compatible with 76 x 25 x 1 mm slides.



- ▣ The antibodies are printed on standard-size microscope slides.
- ▣ Each slide consists of an array of well-characterized antibodies with six replicates.
- ▣ Multiple positive and negative controls are present.
- ▣ Each antibody array kit comes with two identical slides.

### Applications

- ▣ Qualitative Protein Expression Profiling
- ▣ Measure Changes in protein expression level (normal vs. diseased; normal vs. treated)
- ▣ Identify Candidate Biomarkers

### Specifications

- ▣ Spot diameter – 260-280 μm
- ▣ Slide dimensions: 76 x 25 x 1 mm
- ▣ Spot-to-Spot distance 450- 600 μm
- ▣ Barcode label: 23 x 9 mm

### Key Features

- ▣ Each array includes carefully selected antibodies highly relevant for a specific pathway.
- ▣ High-throughput expression profiling using proteins from cell extracts, tissue lysates or treated samples.
- ▣ Less than 5 μg of protein is required for each assay
- ▣ 3D-Polymer coated glass substrates
- ▣ High specificity
- ▣ Low background
- ▣ Sensitive fluorescent detection (Streptavidin – Cy<sup>®</sup>3 (Cy<sup>®</sup>5/Alexa 532/Alexa 647)
- ▣ Six replicates for increased data consistency
- ▣ Suitable for detection on all fluorescent MicroArray scanners compatible with 76 x 25 x 1 mm slides
- ▣ Optimized Antibody Array Assay Kit provides optimized reagents and buffers for protein extraction, labeling, conjugation and detection.
- ▣ GenePix<sup>®</sup> Array List (GAL) files are provided for convenient and accurate data analysis.

### Comprehensive antibody array

| Description                          | Reference  | # Abs | # Slides |
|--------------------------------------|------------|-------|----------|
| Explorer™ Antibody Array             | AR-ASB6-00 | 656   | 2        |
| Phospho Explorer™ Antibody Array     | AR-PEX1-00 | 1318  | 2        |
| Tyrosine phosphorylation proarray AA | AR-PST2-28 | 228   | 2        |

### Pathway-specific arrays

|                                    |            |     |   |
|------------------------------------|------------|-----|---|
| Angiogenesis Antibody Array        | AR-AGE1-25 | 127 | 2 |
| Apoptosis Antibody Array           | AR-APP0-69 | 73  | 2 |
| Cancer Marker Antibody Array       | AR-ACMO-83 | 86  | 2 |
| Cell Cycle Antibody Array          | AR-ACCO-58 | 60  | 2 |
| Cytokine Related Antibody Array    | AR-ACK0-75 | 77  | 2 |
| Hematopoiesis Antibody Array       | AR-AHT0-49 | 49  | 2 |
| Hormone Antibody Array             | AR-AHM0-30 | 30  | 2 |
| Signal Transduction Antibody Array | AR-AST1-60 | 165 | 2 |
| Stem Cell Antibody Array           | AR-ASCO-48 | 58  | 2 |
| Explorer Antibody Array            | AR-ASB6-00 | 656 | 2 |

### Phospho-specific arrays

| Description                               | Reference  | # Abs | # Slides |
|-------------------------------------------|------------|-------|----------|
| AKT Pathway Phospho Antibody Array        | AR-PAA1-37 | 137   | 2        |
| AKT/PKB Signaling Phospho Antibody Array  | AR-PAB2-16 | 216   | 2        |
| Cancer/Apoptosis Phospho Antibody Array   | AR-PAC1-55 | 155   | 2        |
| AMPK Signaling Antibody Array             | AR-PAM1-74 | 174   | 2        |
| Apoptosis Phospho Antibody Array          | AR-PAP2-47 | 247   | 2        |
| Cell Cycle Phospho Antibody Array         | AR-PCC0-76 | 76    | 2        |
| Cell Cycle Control Phospho Antibody array | AR-PCC2-38 | 238   | 2        |
| Cytoskeleton Phospho Antibody Array       | AR-PCP0-95 | 95    | 2        |
| Cytoskeleton II Phospho Antibody Array    | AR-PCP1-41 | 141   | 2        |
| CREB Antibody Array                       | AR-PCR1-74 | 174   | 2        |
| Chromatin/Transcription Phospho AA        | AR-PCT1-73 | 173   | 2        |
| PDGF Antibody Array                       | AR-PDG1-95 | 195   | 2        |
| EGF Pathway Antibody Array                | AR-PEG2-14 | 214   | 2        |
| Erk Signaling Antibody Array              | AR-PEK2-08 | 208   | 2        |
| ErbB/HER Signaling Antibody Array         | AR-PER2-39 | 239   | 2        |
| Phospho Explorer Antibody Array           | AR-PEX1-00 | 1318  | 2        |
| p53 Signaling Antibody Array              | AR-PFT1-96 | 196   | 2        |
| FGF Pathway Antibody Array                | AR-PGF1-56 | 156   | 2        |
| GPCR Signaling to MAPK/ERK Antibody Array | AR-PGP1-93 | 193   | 2        |
| Insulin/Glucose Phospho Antibody Array    | AR-PIG0-85 | 85    | 2        |
| Insulin Receptor Phospho-Specific AA      | AR-PIG2-19 | 219   | 2        |
| IGF-IR Signaling Antibody Array           | AR-PIR2-45 | 245   | 2        |
| Jak/Stat Pathway Phospho Antibody Array   | AR-PJS0-42 | 42    | 2        |
| Jak/Stat II Phospho Antibody Array        | AR-PJS2-02 | 202   | 2        |
| MAPK Pathway Phospho Antibody Array       | AR-PMK1-85 | 185   | 2        |
| mTOR Phospho Antibody Array               | AR-PMT1-38 | 138   | 2        |
| NF-kB Pathway Phospho Antibody Array      | AR-PNK0-63 | 63    | 2        |
| NF-kB II Signaling Phospho Antibody Array | AR-PNK2-15 | 215   | 2        |
| Nuclear/Membrane Receptor Phospho AA      | AR-PNR0-52 | 52    | 2        |
| Neuroscience Phospho Antibody Array       | AR-PNS0-32 | 32    | 2        |
| Tyrosine Phosphorylation ProArray AA      | AR-PST2-28 | 228   | 2        |
| T-Cell Receptor Signaling Antibody Array  | AR-PTC1-88 | 188   | 2        |
| TGF-beta Signaling Antibody Array         | AR-PTG1-76 | 176   | 2        |
| Tyrosine Kinase Adaptor Phospho AA        | AR-PTK0-98 | 98    | 2        |
| VEGF Pathway Antibody Array               | AR-PVE1-85 | 185   | 2        |

**Phospho-specific antibodies are available separately (50-150 μg). Please inquire for reference and price information.**

## Complete Assay Service

We perform one complete antibody array assay with your sample. You can select antibody microarrays to examine normal and treated samples for changes in protein expression and phosphorylation events. This convenient assay service offers quick turnaround, accurate and reliable results, saving you time and resources!

### The protein expression profiling service includes

- ▣ Protein Extraction and labeling
- ▣ Assay condition optimization
- ▣ Conjugation of Biotin Labeled Protein to Antibody Array
- ▣ Detection using Cy<sup>®</sup>3-Streptavidin
- ▣ Scan the arrays on Axon GenePix scanners
- ▣ Data acquisition. (Data provided in Excel format)
- ▣ Turnaround time: usually 8-12 days (maybe longer if there are more than 6 samples).

### Starting material

For each sample you wish to assay, please provide cell or tissue lysates with 400 µg or more total protein at a minimum of 2 µg/µl. Acceptable lysis buffers include Antibody Array Protein Extraction Buffer (AR-EXB-050), RIPA buffer, or other buffers suggested by commonly used lysis protocols. The use of Tris buffer and detergents should be avoided or limited. No more than 50 mM of Tris, 0.20 % SDS, or 1 % non-ionic detergents should be present in the sample. Commonly used protease and/or phosphatase inhibitors may be added to the lysis buffer to preserve the proteins. The proteins in the sample should remain in their native structure (not denatured). In addition to cell or tissue lysates, other suitable samples are:

- ▣ Frozen cell pellets with 5 million cells
- ▣ Frozen tissues, 50-100 mg (usually 75 mg)

## Antibody Array Assay kit

- ▣ The Antibody Array Assay Kit is designed for easy and reliable processing of antibody arrays.
- ▣ Each kit comes with sufficient material and reagents to perform protein extract, labeling, conjugation and detection for two arrays or twenty arrays.
- ▣ The buffers and reagents have been optimized to maximize protein extraction yield, labeling efficiency, and to produce high signal-to-noise ratio.

### Key features

- ▣ Complete and easy-to-use reagent kit
- ▣ Provide major reagents required to process antibody
- ▣ Optimized to work with Thematic, Phospho-specific and Explorer antibody MicroArrays

### Antibody array assay kits

| Description                            | Reference  |
|----------------------------------------|------------|
| Antibody Array Assay Kit, 2 Reactions  | AR-KAS0-02 |
| Antibody Array Assay Kit, 20 Reactions | AR-KAS0-20 |

Speedy 28-day polyclonal  
The new reference  
in immunisation



- ▣ See also Eurogentec custom peptides synthesis service, chapter 3.6