

Finding the Match: 40 Antibody Resources for xMAP[®]-based Multiplex Immunoassays

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The global market for research antibodies is expected to reach \$3 billion in 2019.¹ Selecting the right antibody that works for your specific assay is a daunting process that is often fraught with failure. A Nature article estimates that half of all antibodies are 'bad'.² 'Bad' antibodies are often the product of research that is not reproducible and certainly contributes to wasted research resources.

Good Antibodies Make Great Assays

For the successful use of an antibody on any platform, the method of antibody generation is an important consideration. Monoclonal antibodies are produced from hybridoma cell lines, whereas polyclonal antibodies are harvested from animal serum. Recombinant antibodies are more reliable compared to their monoclonal and polyclonal counterparts because they are made from cultured cell lines carrying an expression plasmid DNA or other expression construct. Other manufacturing parameters used in antibody production to consider include:

- was the antigen native or a denatured protein,
- the sequence uniqueness of the selected protein/antigenic fragment,
- the antibody purification method,
- and the stability of certain antibody preparations.

Each of these factors can significantly impact the reliability, sensitivity, and data quality of an assay.

With multiple variables influencing antibody performance, validation of each reagent for a given application is essential. While many suppliers provide western blotting performance, this is not necessarily an indicator of performance in a multiplex assay. In the western blot, antibodies probe denatured proteins and recognize a linear epitope. In other immunoassay formats, a protein's native structure might hide a linear epitope and prevent the antibody from recognizing its target sequence. A recent article in Nature Methods by the International Working Group on Antibody Validation details five pillars to consider for reliable antibody selection and validation: genetic strategies, orthogonal strategies, independent antibody strategies, tagged proteins, and immunocapture-mass spectrometry.³ Before purchasing your next batch of antibodies, consider asking your supplier for validation data in addition to what is provided in the product information.

Immunoassay Applications

Antibodies are the foundation of traditional immunoassays such as ELISAs and Western Blots, but these formats lack throughput. In contrast, Luminex's xMAP[®] Technology provides flexible immunoassay formats that support:

- Capture sandwich
- Competitive
- Indirect serological multiplex assays
- Protein interaction assays

xMAP-based assays can enable the testing of up to 500 analytes in a single sample reaction. xMAP Technology uses color-coded microspheres that can be coated with protein or nucleic acid capture molecules. Spectrally distinct sets of microspheres allow for the simultaneous capture of multiple analytes from a single reaction. Because of the small size of the microsphere and low density, xMAP assays exhibit virtual solution-phase kinetics during the reaction. However, during analysis, the solid phase microspheres permit each bead to be analyzed discretely. Whether you buy a pre-configured kit from a Luminex Partner or choose to build your own immunoassay, good antibodies are the foundation of great assays.

Buy It: Off the Shelf Kits and Custom Assay Solutions

The **xMAP[®] Kit Finder** has a robust selection of research immunoassays that are offered by over 60 **Luminex Partners**. Not finding what you are looking for? The following Partners provide customizable assay and reagent solutions:

- ThermoFisher Scientific: **ProcartaPlex[®] Panel Configurator**
- Bio-Rad: **Bio-Plex[®] Assay Builder**
- EMD Millipore: **Custom Assay Development Services**
- Origene: **Reagent and Kit Development**
- R&D Systems a BioTechnique[®] Brand: **Luminex Assay Customization Tool**
- Radix BioSolutions: **Custom Reagent and Assay Services**

Build It: Sources for Proteomic Assay Reagents

Do you want to develop a custom xMAP-based proteomic assay yourself but lack the resources to make your own reagents? Finding the right combination of commercial or publically available antibodies or proteins can be challenging, but selecting the right combination of antibodies and control proteins is critical for optimum assay performance.

Pivotal Scientific provides a listing of the 318 most popular commercial antibody suppliers ranked by page views if you are looking to identify new vendors.

Of all the antibody sources, **eBioscience**® now part of **ThermoFisher** and **R&D Systems, a BioTechne Brand** provide compatibility information for antibody pairs on the xMAP platform.

Novus Biologicals®, a **BioTechne Brand** and **OriGene**™ have validated their antibodies on the Luminex platform and have developed xMAP-based assays.

NIH Sponsored Resources

- **AbMiner**: database of over 600 commercially available antibodies that are matched to their respective genomic identifiers
- **Antibody Portal**: National Cancer Institute resource with approximately 400 antibodies available for purchase
- **BEI Resources**: provides reagents at no cost for priority pathogens, emerging infectious disease agents, and non-pathogenic microbes
- **Chemical Biology Laboratory Database of Anti-Glycan Reagents**: contains more than 1,100 antibodies and lectins
- **Developmental Studies Hybridoma Bank**: sells more than 3,700 antibodies at cost
- **NeuroMab**: generates novel monoclonal antibodies for neuroscience and other fields of basic biomedical research
- **Nonhuman Primate Reagent Resource**: produces conventional antibodies for nonhuman primate in vitro diagnostics, as well as primatized recombinant antibodies and fusion proteins for in vivo administration

Sourcing from Multiple Vendors

When an assay requires antibodies (and control proteins) sourced from multiple vendors, the websites in Table 1 are good resources.

These sites provide information about antibodies from commercial suppliers that pay for listing on these sites. As a result, any platform-specific information about a company's antibodies is provided by the reagent supplier and may not be noted by the resource website. Of these sites, only **Linscott's Directory** and **Antibodies-online** lists when Luminex has been used. The **BioCompare** site denotes antibody compatibility on multiplex platforms with "Mpx".

In addition to the sources listed above, **this Nature article** provides an extensive table of antibody manufacturers, suppliers, and immunoassay developers.⁴

Table 1

Website	Application	Publications	Validation	User Reviews	Comparisons
Antibody Directory™	●				
Antibodies-online	●	●	●		
Antibody Resource	●				●
Antibody Registry					
BioCompare	●			●	
GenLogica	●	●	●		
iSpyBio	●	●	●	●	●
Labome	●	●			
Linscott's Directory	●			●	
SeekSequence	●	●			●
SelectScience	●			●	
1DegreeBio	●			●	

Unconventional Capture and Detection Molecules

Camelid (camels, alpacas, llamas) single domain antibodies are devoid of light chains and are often called **heavy chain antibodies**. Previous studies on the Luminex platform have used their unique properties to measure levels of botulinum toxin⁵, measure antibody titers to viral proteins⁶, and to study protein-protein interactions.⁷ Vendors such as **Allele Biotech**, **Capralogics**, and **ChromoTek** offer camelid antibodies.

Non-antibody molecules like aptamers⁸ and Slow Off-rate Modified Aptamers (SOMAmers®)⁹ have also been used on the Luminex platform to develop capture sandwich assays. Aptamers are RNA or ssDNA that are 15–60 nucleotides in length and bind with affinity in the nanomolar range. **Aptagen** offers a robust database of aptamers as well as custom design services. SOMAmers are modified DNA aptamers with nano- to pico-molar affinity and high specificity for their cognate analytes. **SomaLogic** provides custom SOMAmer reagents and assay development services.

User Reviews

With the abundance of antibodies available and the lack of negative results in published literature, identifying new antibodies can be quite challenging. Before selecting your next batch of new reagents, leverage the experience of immunoassay community from these non-commercial websites:

CiteAb

CiteAb is a citation-ranked antibody search tool hosted by the University of Bath. It collects information from commercial suppliers and non-commercial sources and also uses information collected from peer-reviewed journals to rank a reagent's use by the scientific

community. When antibodies are available from multiple suppliers, the site ranks them by the number of research articles that references using the reagent. CiteAb also indicates what platforms the antibodies have been used on, including Luminex, when this information is provided by the supplier or indicated in published papers.

pAbmAbs

The **pAbmAbs** site enables users to search for antibodies against different proteins or small molecules and to post reviews about utility for a broad range of applications (western blotting, immunostaining, immunoprecipitation, ELISA, flow cytometry, and the xMAP Technology). Simply enter the name of the target protein and the application to find reviews. Users can post reviews about the performance of antibodies they have used and can rate an antibody's utility on the different platforms.

Antibodypedia

Antibodypedia is a searchable resource reporting primary data, publications, and commentary on publicly available antibodies which detect human protein targets. Antibody validation data is structured in an application-specific manner and antibodies are given a score based on the knowledge associated with them. You can **submit your data** for inclusion on the website according to the guidelines proposed by the International Working Group on Antibody Validation.

Optimize and Validate Your Assay

Once you have identified candidate antibodies for your custom multiplex assay, it is time to test the antibodies on the xMAP

platform. Singleplex testing establishes the best capture and detection antibody pairs for each analyte. Once these pairs are identified with the singleplex assays, the next step is to evaluate the compatibility of all the capture beads and detection antibodies in the same multiplex reaction environment.

Before testing the effectiveness of the multiplex reaction, the singleplex testing of potential capture and detection antibody pairs often requires testing antibodies from several different sources for compatibility. Lawson et. al. developed an eight-plex (IL-1 β , IL-8, IFN- α , TNF- α , IL-12, IL-10, IFN- γ , and IL-4) porcine cytokine assay using specific capture and detection antibodies from six different commercial sources.¹⁰ The authors reported the final set of 15 antibodies used, but they tested for 30 different antibodies from nine different sources before finding the optimal combination used in the final multiplex assay (J. Lunney, personal communication). Higher plex assays require screening even more antibodies. Skogstrand et al. developed several inflammatory marker assays from 24 to 26 plex that required screening more than 100 antibodies from several manufacturers before finding the best capture/detection pairs.^{11,12}

We are Here to Help!

Instructions for coupling and testing different combinations of antibodies can be found in the **xMAP Cookbook** and **Dunbar and Hoffmeyer**. Additional tips can be found on the **Luminex blog** or by contacting your **Luminex Field Application Scientist**.

References

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Off The Shelf Kits and Custom Assay Solutions

Bio-Rad: Bio-Plex® Assay Builder	http://www.bio-rad.com/en-us/product/bio-plex-assay-builder
EMD Millipore: Custom Assay Development Services	http://www.emdmillipore.com/US/en/life-science-research/protein-detection-quantification/Immunoassay-Platform-Solutions/Custom-Assay-Development-Services/4y2b.qB.uuIAAFLn50Jx1Br,nav
Luminex Partners	https://www.luminexcorp.com/partners/life-science/
Origene: Reagent and Kit Development	http://www.origene.com/Assays/Development.aspx
Radix Biosolutions: Custom Reagent and Assay Services	http://www.radixbiosolutions.com/
R&D Systems a BioTechne Brand: Luminex Assay Customization Tool	https://www.rndsystems.com/luminex/analytes
ThermoFisher Scientific: ProcartaPlex® Panel Configurator	http://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-assays-analysis/luminex-multiplex-assays/procartaplex-assays.html

Build It: Sources For Proteomic Assay Reagents

eBioscience now part of ThermoFisher	http://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-assays-analysis/luminex-multiplex-assays/procartaplex-assays.html
Pivotal Scientific	http://www.pivotalscientific.com/antibody-suppliers-ranking
R&D Systems, a BioTechne Brand	https://www.rndsystems.com/

NIH Sponsored Resources

AbMiner	https://discover.nci.nih.gov/abminer/home.do
Antibody Portal	https://antibodies.cancer.gov/apps/site/default
BEI Resources	https://www.beiresources.org/
Chemical Biology Laboratory Database of Anti-Glycan Reagents	https://ccr2.cancer.gov/resources/Cbl/Tools/Antibody/
Developmental Studies Hybridoma Bank	http://dshb.biology.uiowa.edu/
NeuroMab	http://neuromab.ucdavis.edu/
Nonhuman Primate Reagent Resource	http://www.nhpreagents.org/NHP/default.aspx

Sourcing from Multiple Vendors

Antibody Directory	http://www.antibodydirectory.com/
Antibodies-online	http://www.antibodies-online.com/
Antibody Resource	https://www.antibodyresource.com/
Antibody Registry	http://antibodyregistry.org/
Biocompare	http://www.biocompare.com/
Genlogica	http://www.genlogica.com/
iSpyBio	http://www.ispybio.com/
Labome	https://www.labome.com/
Linscott's Directory	https://www.linscottsdirectory.com/
SeekQuence	http://www.seekquence.com/
SelectScience	http://www.selectscience.net/
1DegreeBio	http://1degreebio.org/

Unconventional Capture and Detection Molecules

Allele Biotech	http://www.allelebiotech.com/camelid-antibody-development/
Capralogics	https://www.capralogics.com/llama-antibodies
ChromoTek	http://www.chromotek.com/home-of-alpaca-antibodies/
Aptagen	http://www.aptagen.com/home.aspx
SomaLogic	http://www.somallogic.com/

User Reviews

CiteAb	https://www.citeab.com/
pAbmAbs	http://pabmabs.com/wordpress/
Antibodypedia	https://www.antibodypedia.com/

Luminex Resources

Luminex Blog	https://www.luminexcorp.com/blog/
Luminex's Field Application Scientists	https://www.luminexcorp.com/support/
Luminex Publications Database	https://www.luminexcorp.com/publications/
xMAP Cookbook	http://info.luminexcorp.com/en-us/download-the-xmap-cookbook
xMAP® Kit Finder	https://kitfinder.luminexcorp.com/

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