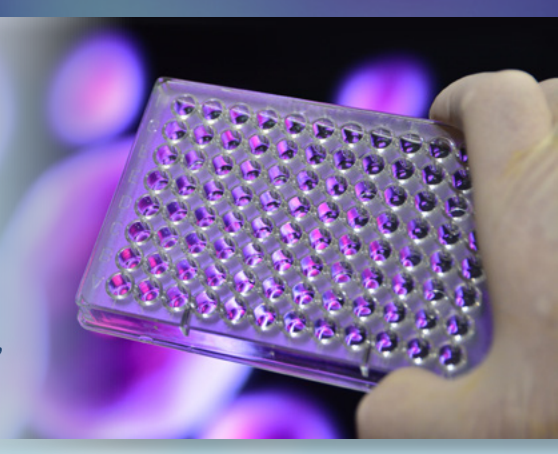
Guide to Multiplex Immunoassays

Multiplex immunoassays measure multiple analytes in a single sample, offering considerable advantages over traditional ELISAs. These assays are commonly used in research and increasingly clinical diagnostics. They provide high analytical accuracy, save time, material, and labor costs, and allow efficient handling of a large number of samples. This infographic will cover some of the most popular multiplex immunoassay platforms, tips for platform selection, the benefits of multiplexing, and best practices for optimizing workflows.

Multiplex Platforms

The number of multiplex platforms continues to grow, but the five systems highlighted here are among the most commonly used.



Platform: Antibody arrays from RayBiotech

Level of plex possible: 8000 proteins (semi-quantitative) or 1400 proteins (quantitative)

Compatible samples types: Any biological fluid, cell culture medium, cell extract, or tissue lysate

Instrumentation required: Compatible with any standard microarray scanner, CCD camera/x-ray film developer, or flow cytometer with red and blue lasers depending on the chosen platform

Platform: Luminex xMAP® Technology

Level of plex possible: Up to 500 proteomic or genomic biomarkers

Compatible samples types: For proteomic biomarkers: clinical biospecimens, cell culture supernatants, and non-conventional matrices such as wound dressings, air/breath, and dried-blood spots. For genomic biomarkers: FFPE tissue, liquid biopsy material, and conventional biospecimens

Instrumentation required: Luminex fluorescence analyzer and xMAP microspheres

Platform: SignalStar™ Multiplex IHC from Cell Signaling Technology

Level of plex possible: 8

Compatible samples types: FFPE tissue

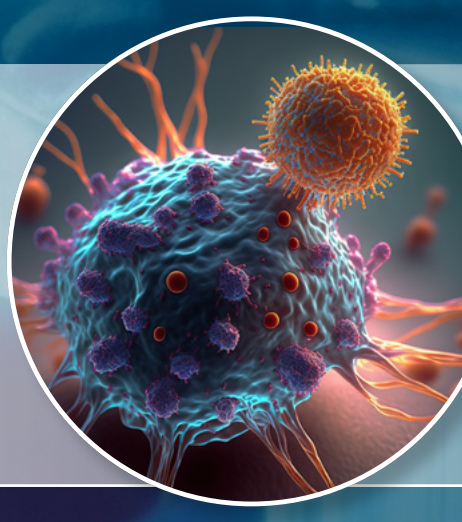
Instrumentation required: Protocols can be run manually at the lab bench or automated on the BOND RX Autostainer by Leica Biosystems; images can be captured with most fluorescent imaging microscopes

Platform: Ella™ instrument and Simple Plex Assays™—automated ELISA—from Bio-Techne

Level of plex possible: 8

Compatible samples types: Complex samples including serum, plasma, and cell culture supernatants

Instrumentation required: Ella instrument and Simple Plex cartridges



Platform: Simple Western—automated western blotting—from Bio-Techne

Level of plex possible: Varies by assay; Multichannel detection of 6 targets plus total protein detection in 8 samples with 1 run has been demonstrated

Compatible samples types: Complex samples including cell, viral, and tissue lysates

Instrumentation required: Simple Western instrument and capillaries

Tips for Choosing the Best Platform

Each platform has its own strengths and weaknesses and the best choice will depend on the questions being asked. Factors to consider include:

- Type of research and number of analytes to be measured
- Expected concentration range of the analytes to be investigated
- The ability of the platform to accurately measure the desired analytes without cross-reactivity
- The volume of sample required for each assay
- The ability to customize panel design
- Ease of use, cost, and throughput
- Technical and scientific support offered by vendors

Benefits of Multiplex Immunoassays

- Smaller sample volumes
- Less hands-on time
- Reduced cost per data point
- Ease of development
- Opportunities for automation
- More results per sample
- Improved experimental consistency
- Customizable to include only relevant targets

Best Practices for Developing and Running a Multiplex Immunoassay

- Understand the biology of your target analytes (e.g., concentration range, pH/temperature sensitivity, stability over time)
- Be consistent with your sample preparation technique
- Validate any reagent or kits with your own model system before testing precious samples
- Avoid unwanted cross-reactivities
- Where fluorescent readouts will be used, prevent spillover that could produce misleading results
- Establish quality control metrics to ensure results are consistent over time

References

- Monoplex and Multiplex Immunoassays: Approval, Advancements, and Alternatives
- Multiplex Assay Selection and Optimization
- Multiplex Platform Review and Selection Guide
- Selecting a Multiplex Platform
- Types of Multiplex Immunoassays