

# A bead-based multiplex immunoassay for cancer autoantibody biomarker discovery

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**An Important Update:**  
MILLIPLEX® MAP Human Cancer Autoantibody Panel (Cat. No. [HCABMAG-13K](#)) is now available as a 15-plex multiplex kit. This poster was originally presented at AAI 2018. You can find more information on this 15-plex panel at: [www.emdmillipore.com/milliplex](http://www.emdmillipore.com/milliplex).

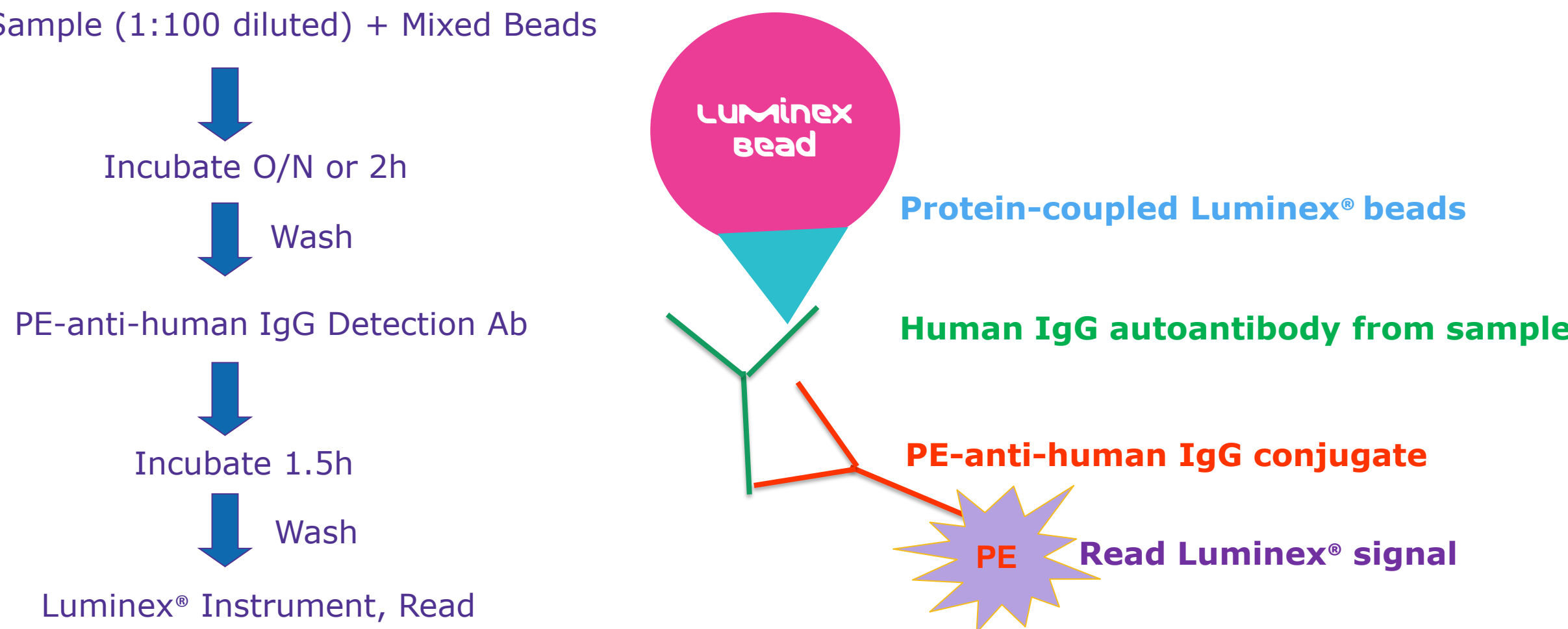
## Abstract

Accurate, reliable and affordable blood tests are needed for early detection of cancer. Here we show data for the development and validation of a sensitive Luminex® bead-based multiplex immunoassay for detecting cancer autoantibodies in multiple cancer types. The cancer autoantibody biomarkers in this new multiplex panel include: CTAG1B/NY-ESO-1, Cyclin B/CCNB1, ENO1, Galectin-1, Galectin-3, Her2/ErbB2, HIF1α, HSP60, IMP2/IGF2BP2/p62, IMP3/IGF2BP3/KOC, MUC1, p16-INK4a, p53, SOX-2, and Survivin/BIRC5. This multiplex immunoassay is sensitive and robust. In a 96-well plate, a mixture of recombinant protein-immobilized magnetic bead sets was incubated with serum samples for 2 hours with gentle shaking; after washing, the beads were incubated with detection antibody phycoerythrin (PE)-labeled anti-human IgG for 1.5 hours. After the final wash, the beads were resuspended in sheath fluid and the median fluorescent intensity (MFI) data of 50 beads per bead set were analyzed on a Luminex® reader. The performance of this panel was demonstrated using a set of serum samples from 78 cancer cases and 18 healthy controls. Elevated cancer autoantibodies were detected in serum from patients with various cancer types, as compared to healthy controls. In conclusion, this cancer autoantibody multiplex immunoassay has potential application as a non-invasive tool for studying early detection, diagnosis or monitoring of cancer.

## Methods

**Samples:** Human serum samples were purchased from Discovery Life Sciences, Inc. and BioIVT, including samples from patients with breast cancer, colorectal cancer, ovarian cancer, lung cancer, prostate cancer and some other cancer types and healthy control serum samples.

### Cancer Autoantibody Multiplex Assay Kit Protocol:



**Cancer Autoantibody Multiplex Immunoassays:** Cancer autoantibody profiles were determined using MILLIPLEX® MAP Cancer Autoantibody immunoassay alpha-kits. Sera were tested at 1:100 dilution in kit assay buffer. Samples were analyzed on a Luminex® 200™ System.

Luminex® 200® System	FLEXMAP 3D® System	MAGPIX® System
<ul style="list-style-type: none"><li>• Multiplex: 100 analytes</li><li>• Flexible: Mag &amp; non-mag</li><li>• User-friendly</li><li>• Scalable: Bar-code reader</li></ul>	<ul style="list-style-type: none"><li>• Highest multiplex: 500</li><li>• 96- and 384-well</li><li>• Ultra-fast: &lt;1 hour</li><li>• Automation / LIS</li></ul>	<ul style="list-style-type: none"><li>• Low-cost: 50 analytes</li><li>• CCD imaging</li><li>• Easy to use</li><li>• Small footprint</li></ul>

## Results

Figure 1. Detection of cancer autoantibodies in serum samples using a 15-plex multiplex assay

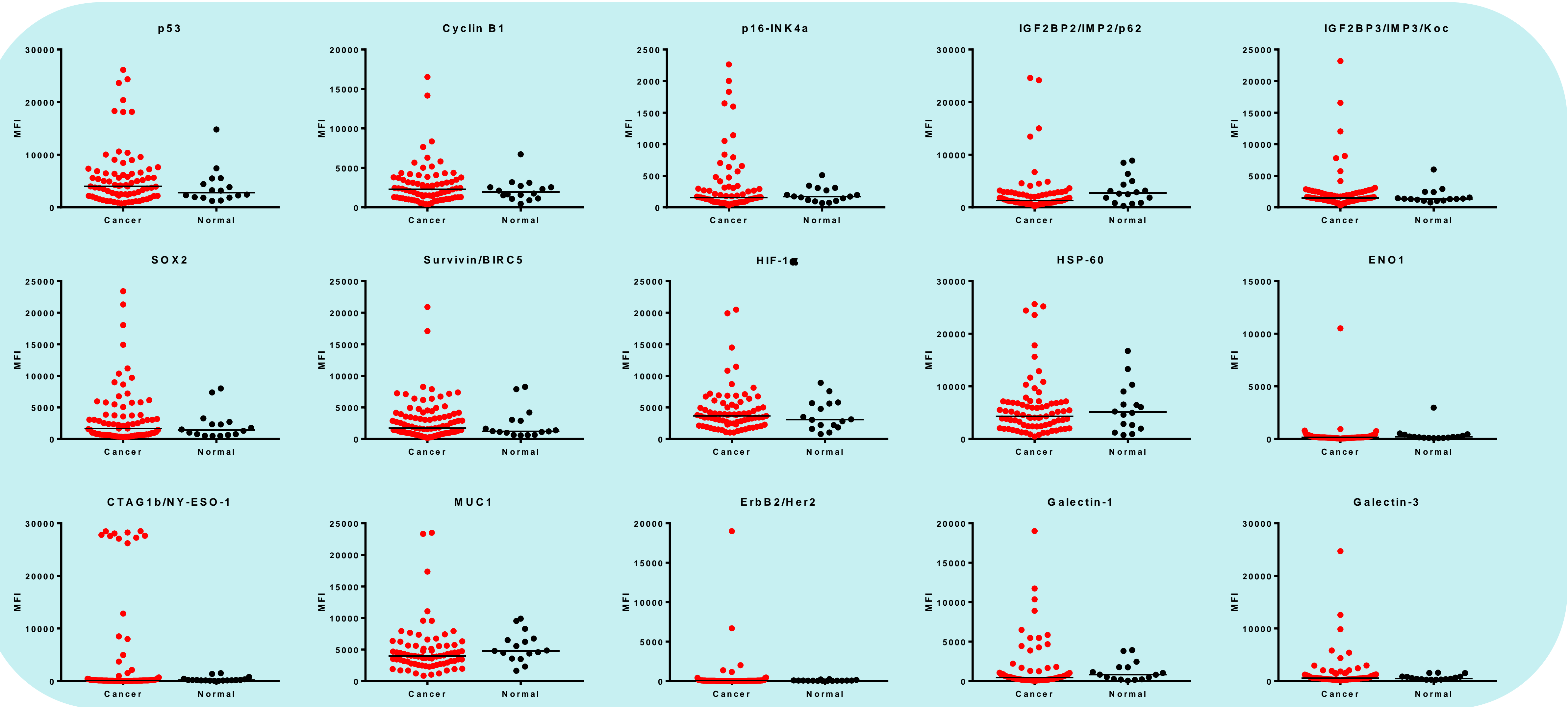


Figure 2. Test specificity of the multiplex assay using multiplex beads and single-plex biotinylated detection antibody

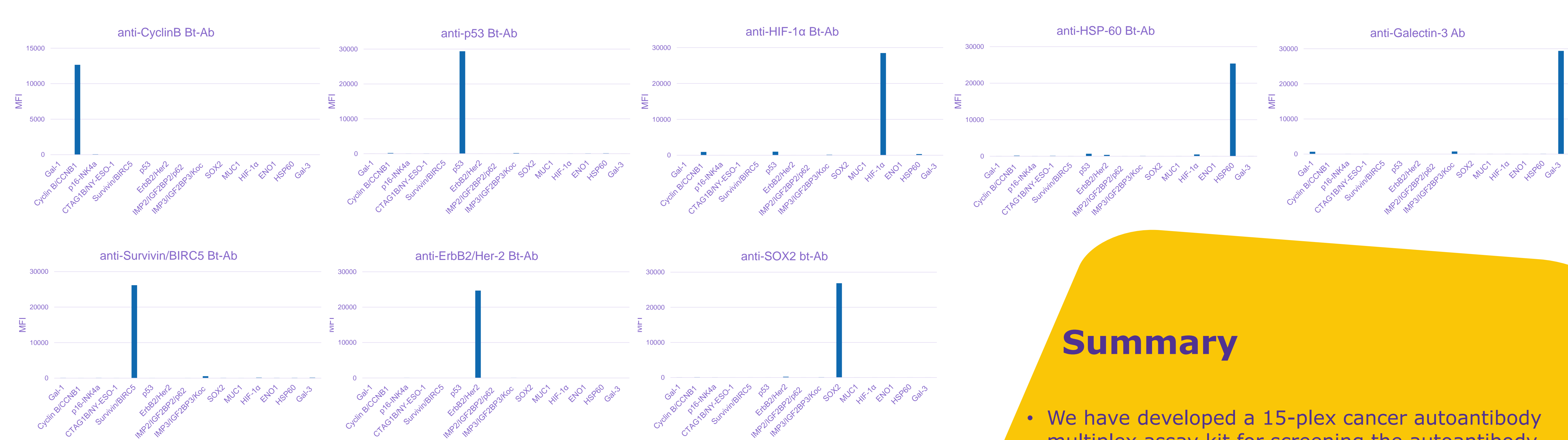
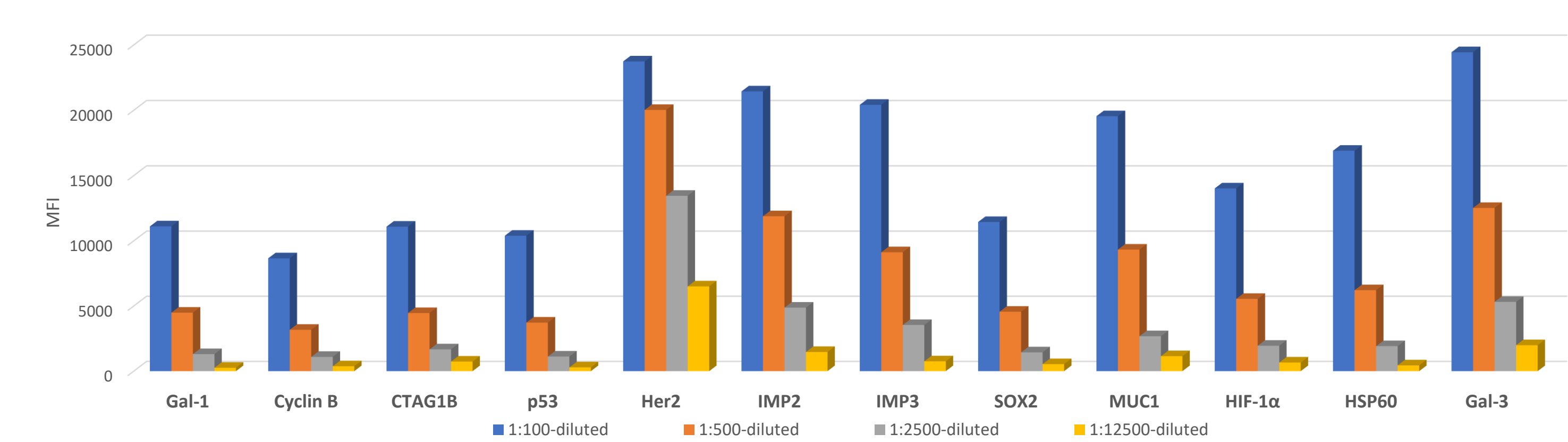


Figure 3. Reactivity of serial diluted serum cancer autoantibody



## Summary

- We have developed a 15-plex cancer autoantibody multiplex assay kit for screening the autoantibody profile in patients with various cancer types.
- Individual tumor-associated protein was coupled to a set of Luminex® beads, and assay specificity was validated by demonstrating the protein-bead reactivity to its specific detection antibody in a multiplex bead setting.
- The cancer autoantibody multiplex assay is robust; it requires less than 5µL of human serum samples, as the sera were tested at 1:100 dilution.
- This human cancer autoantibody multiplex assay can be used alone, or in combination with MILLIPLEX® MAP cancer circulating biomarker panels, as useful biomarker screening tools in studying early detection of cancer.

[www.emdmillipore.com/milliplex](http://www.emdmillipore.com/milliplex)