Novel Formulation for Eliminating Non-Specific Binding to Microspheres in Multiplexed Luminex Assays and Other IVD Applications

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The 7th Annual Planet xMAP Multiplexing Symposium 2010

Abstract

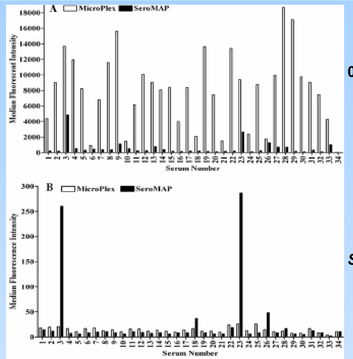
Recent studies have shown that incorporating StabilGuard® BSA-Free Immunoassay Stabilizer/Blocker (product code SG01) during both the washing and blocking steps greatly reduced non-specific reactivity to microspheres in assays based on Luminex® xMAP technology. These types of interferences demonstrated unique assay issues that had significant negative impacts on assay reliability. ARUP Laboratories (Salt Lake City, UT), has indicated false-positive issues in their pneumococcal antibody assay due to human sera non-specifically binding directly to the microspheres as well as specifically binding to BSA (Pickering, J. W., 2010, *Clin. Vaccine Immunol.*, CVI.00329-09). Merck Research Laboratories (Wayne, PA), has indicated similar issues in their multiplexed human papillomavirus (HPV) immunoassay (Opalka, D., 2010, *Clin. Vaccine Immunol.*, CVI.00348-09). Both labs have shown that the addition of SG01 at the blocking step in the procedure reduced the non-specific binding of the sera to the microspheres, including an impressive 99.7% reduction in the ARUP assay. Both labs demonstrated the addition of SG01 eliminated the polyspecific reactivity to BSA and improved assay sensitivity. Merck also demonstrated that the use of SG01 as the blocking buffer allowed the authors to decrease the microsphere concentration within the assay and improved lot-to-lot consistency of the microspheres. SurModics' novel formulation has broad applicability across the immunoassay diagnostic industry. SG01 is best known for dried protein stability, demonstrating greater than 85% retained antibody activity after 30 months at room temperature. SG01 as an immunoassay blocker has demonstrated superior coating and uniformity suggesting optimal blocking effectiveness in polystyrene plates, microspheres, membranes, and microarray slides. Many other successful applications of SG01 include, use as an assay diluent, heterophilic blocker, in-solution protein storage buffer and suspension buffer preventing microsphere aggregation. For almost 20 years, SG01 has remained the gold standard as a BSA-free immunoassay stabilizer/blocker. While *StabilGuard* Immunoassay Stabilizer/Blocker has exhibited a long history of improved diagnostic immunoassay performance, these studies demonstrate that the same formulation technology can be transferred into next generation diagnostics, including multiplexed assays based on *Luminex* xMAP technology.

Objectives/Goals

Demonstrate the benefits of *StabilGuard* Stabilizer/Blocker (SG01) for use in multiplexed *Luminex* assays as a microsphere blocker and wash buffer. Also demonstrate *StabilGuard* Stabilizer/Blocker's many other benefits across the immunoassay diagnostic industry.

Microsphere Applications

Figure #1: *StabilGuard* Blocker's elimination of nonspecific reactivity of human sera to uncoupled microspheres



0.1% BSA/PBS Buffer

***StabilGuard* Blocker**

Figure #1: Used with permission from the American Society for Microbiology

Methods: MicroPlex® (clear bars) and SeroMAP® (solid bars) microspheres were resuspended in (A) 0.1%BSA/PBS buffer and (B) *StabilGuard* Blocker. Thirty-three serum samples exhibiting very high false positive results and one control serum sample (#34) were incubated with the uncoupled microspheres for 20 minutes at room temperature with shaking. See (Pickering, J. W., 2010, Clin. Vaccine Immunol., CVI.00329-09) for the remainder of the assay procedure.

Results: Median fluorescence intensities (MFI) for the 33 serum samples previously exhibiting non-specific reactivity are shown in Figure #1 above. All of the 33 sera tested reacted strongly to the *MicroPlex* (clear bars) microspheres in (A) 0.1%BSA/PBS buffer. Nonspecific binding to *MicroPlex* (clear bars) microspheres was completely eliminated when suspended in (B) *StabilGuard* Blocker (reduced by 99.7%). With the exception of two serum samples, *StabilGuard* Blocker also eliminated the non-specific binding to the *SeroMAP* (solid bars) microspheres, still demonstrating an overall dramatic MFI reduction with *StabilGuard* Stabilizer/Blocker.

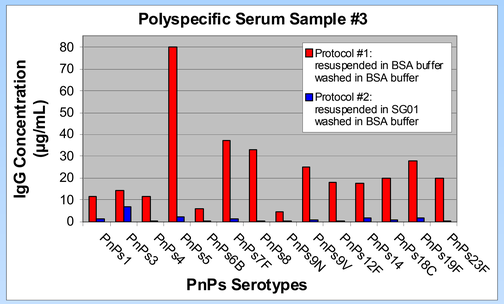


Figure #2: SG01's removal of nonspecific binding to coupled microspheres

Methods: Protocol #1: *MicroPlex* microspheres coupled to pneumococcal polysaccharides (PnPs) were washed, blocked and resuspended in 0.1%BSA/PBS buffer. Protocol #2: coupled *MicroPlex* microspheres were resuspended in *StabilGuard* Blocker instead of 0.1%BSA/PBS buffer.

Results: Figure #2 above shows one serum sample which previously demonstrated very high levels of nonspecific reactivity to *MicroPlex* microspheres. Upon the introduction of *StabilGuard* Stabilizer as the microsphere resuspension buffer in protocol #2, the polyspecific reactivity of the sera to the microspheres was eliminated.

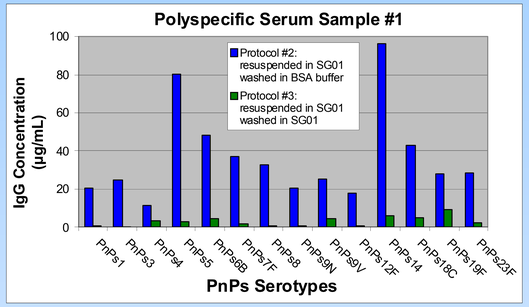


Figure #3: Removal of false positives due to BSA reactivity using SG01

Methods: A subset of sera samples remained which demonstrated false positive reactivity. These sera reacted strongly with microspheres coated with BSA. Protocol #2 was further modified by removing the 0.1% BSA/PBS wash step. Protocol #3 resuspended the microspheres with SG01 and also washed and blocked the microspheres with SG01.

Results: Figure #3 above shows one of two serum samples which previously demonstrated reactivity to BSA. Upon the removal of BSA from the blocking and wash steps and replacing with *StabilGuard* Blocker (BSA-free) as the microsphere wash/block buffer in protocol #3, the false positive reactivity of the sera to BSA was eliminated.

Enhanced Polyclonal Antibody Stability

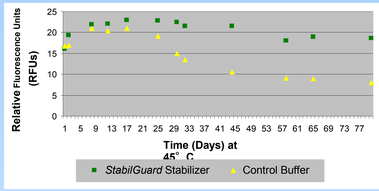


Figure #4: *StabilGuard* Stabilizer as a microsphere stabilizer and blocker

Increased Colloidal Stability

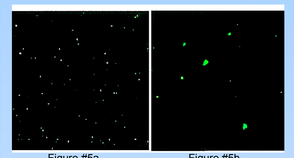


Figure #5a Microspheres in SG01

Figure #5b Microspheres in control buffer

Figure #5: In-solution colloidal stability of microspheres with *StabilGuard* Stabilizer

Methods: Accelerated and real-time stability studies were conducted to evaluate the effectiveness of *StabilGuard* Stabilizer/Blocker to block and stabilize as well as to maintain colloidal stability of polystyrene microspheres in solution. A rabbit polyclonal antibody was covalently attached to polystyrene microspheres. The antibody-coated microspheres were stabilized with either *StabilGuard* Immunoassay Stabilizer/Blocker or a PBS control buffer and stored in solution at 45°C.

Results: Compared to the PBS control buffer results, which showed that rabbit polyclonal antibody-coated microspheres dropped below 90% binding activity after 29 days at 45°C, Figure #4 demonstrates *StabilGuard* Stabilizer retained >96% binding activity after 79 days at 45°C. The colloidal stability of *StabilGuard* Blocker versus a control buffer is illustrated in Figure #5. The PBS control buffer image (Figure #5b) demonstrates aggregation of microspheres in solution, while *StabilGuard* Blocker (Figure #5a) prevents aggregation leading to increased assay precision and accuracy.

Immunoassay Protein Stabilization and Blocking Applications

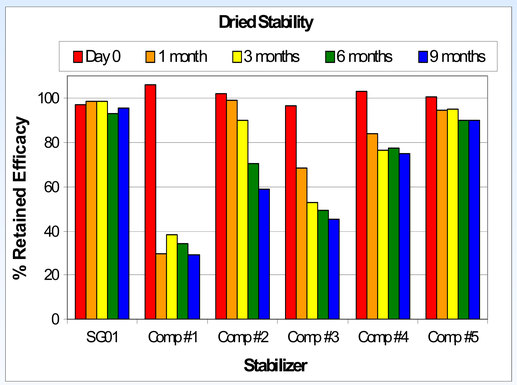


Figure #6: Dried Antibody Stability with *StabilGuard* Stabilizer

Methods: A capture antibody was coated and stabilized with different commercially available BSA-free stabilizers. This study accelerated the stability conditions by challenging the captured antibody at a 37°C storage condition, versus a 4°C control. The retained activity of the captured antibody was evaluated in a sandwich ELISA over nine months by comparing the immunoassay signal produced at 4°C versus 37°C.

$$\% \text{ Retained Activity} = \frac{\text{Average OD @ 37°C}}{\text{Average OD @ 4°C}} \times 100$$

Results: At the nine month stability time point, *StabilGuard* Stabilizer demonstrated greater than 90% retained activity. The sustained functional activity suggests *StabilGuard* Stabilizer was able to preserve the functional conformation of the dried antibody. Most other competitors demonstrate a dramatic decrease in retained antibody activity over the same accelerated conditions.

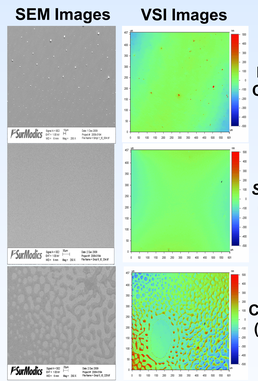


Figure #7: Blocking Uniformity with *StabilGuard* Blocker

Methods: An antibody was coated onto a polystyrene ELISA plate, incubated, washed and blocked with *StabilGuard* Blocker (BSA-free), a BSA-free competitor or left blank. The blockers were incubated and dried at low humidity (<15%). Scanning Electron Microscopy (SEM) and VSI (Vertical Scanning Interferometry) surface characterization imaging were performed to demonstrate each product's ability to completely block a polystyrene plate and prevent an antibody from binding to the blocked surface.

Results: *StabilGuard* Blocker demonstrates superior coating and uniformity suggesting optimal blocking effectiveness. The competitor images demonstrate a non-uniform coating leading to inconsistent blocking and increased nonspecific binding.

Primary (Gt x rbGAPDH) – 1:4000
Secondary (Dk x Gt-IgG-HRP) – 1:20,000

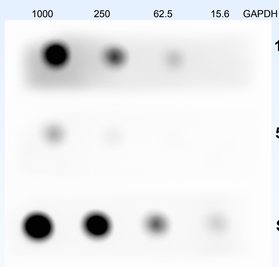


Figure #8: *StabilGuard* Blocker as a membrane blocker

Methods: GAPDH was absorbed to a nitrocellulose membrane and blocked with one of three blocking solutions: 1% BSA in TBS, 5% milk in TBS, or *StabilGuard* Blocker. After washing of the secondary antibody the membranes were developed using chemiluminescent substrate, placed on a glass plate and imaged using a CCD camera

Results: The dot blot to the left demonstrates the use of *StabilGuard* Blocker as the membrane blocker provided strong blocking, decreased backgrounds, and also enhanced detection limits.

Microarray Applications

Enhanced Signal Intensity in Antibody Microarrays

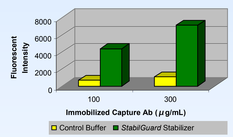


Figure #9: Microarray applications with *StabilGuard* Blocker

Methods: Monoclonal antibody to IFNγ (100 and 300 µg/mL) was arrayed on a SurModics protein-binding surface using BioRobotics split pins and a BioRobotics arrayer. Cocktail antigens containing recombinant human IFNγ at 25 ng/mL were incubated on slides using a PBS control buffer and *StabilGuard* Blocker. After incubation, arrays were developed with biotinylated antibody cocktail suspended in the same reagents, respectively. The spots were visualized by incubating with Streptavidin Cy5.

Results: Figure #9 demonstrates the use of *StabilGuard* Blocker as an antigen dilution buffer dramatically increased the immunoassay signal.

Summary – *StabilGuard* Stabilizer/Blocker Features:

- **Reduced the non-specific binding of sera to microspheres in multiplex *Luminex* assays, including an impressive 99.7% reduction in the ARUP assay**
- **Eliminated sera reactivity to BSA when substituted during the microsphere wash steps**
- **Decreased microsphere concentration and improved lot-to-lot consistency of the microspheres leading to improved assay sensitivity**
- **Provided superior dried antibody stability**
- **Provided strong blocking, decreased backgrounds, and enhanced detection limits with membrane based immunoassays**
- **Demonstrated improved assay performance in multiple applications across the immunoassay diagnostic industry**

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