The Antifouling Properties of BSA on Magnetic Beads are Affected by BSA Grade, Bead Type, and pH



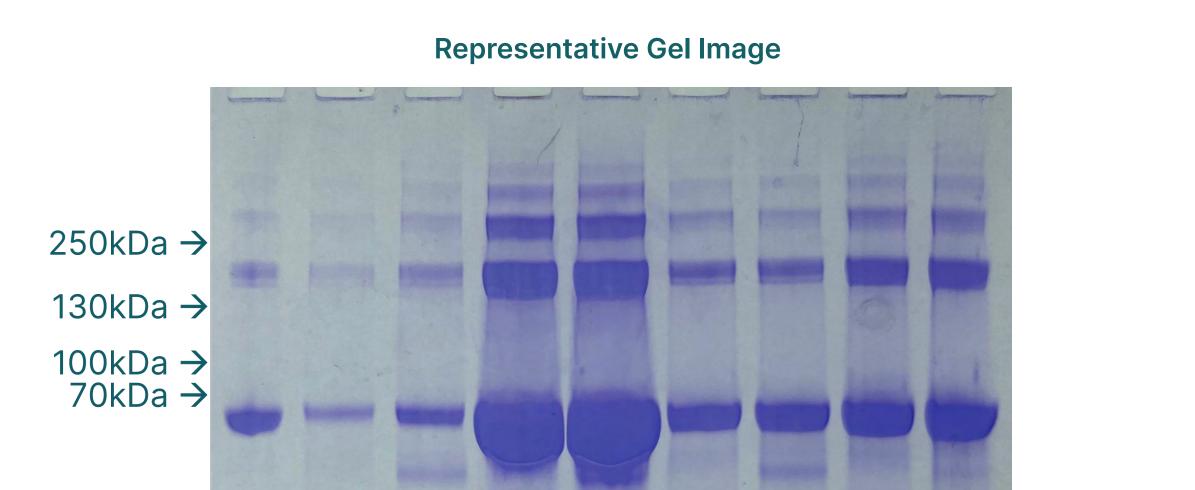
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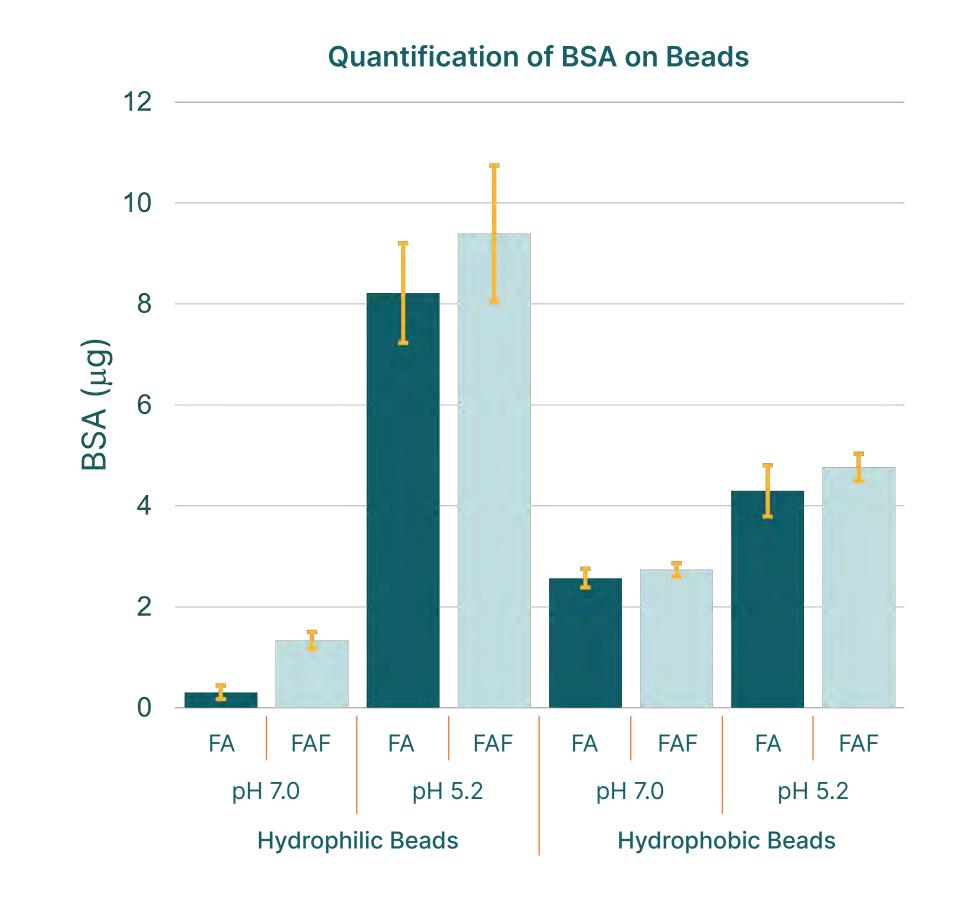
Background

BSA (bovine serum albumin) is a protein that is widely used to increase the sensitivity of immunoassays by coating surfaces and blocking nonspecific interactions, thereby lowering background noise. One such immunoassay is the bead-based ELISA (enzyme-linked immunosorbent assay), which can provide quantitative detection of many diverse analytes. The BSA grade chosen for an assay is frequently based on experience, as there is a lack of understanding of how BSA grades interact with different bead surfaces under various assay conditions. The objective of this study is to examine if different BSA grades work better with particular bead chemistries and how pH influences those interactions by quantifying the amount of BSA on ELISA beads and assessing the blocking of nonspecific antibody signal.

Results

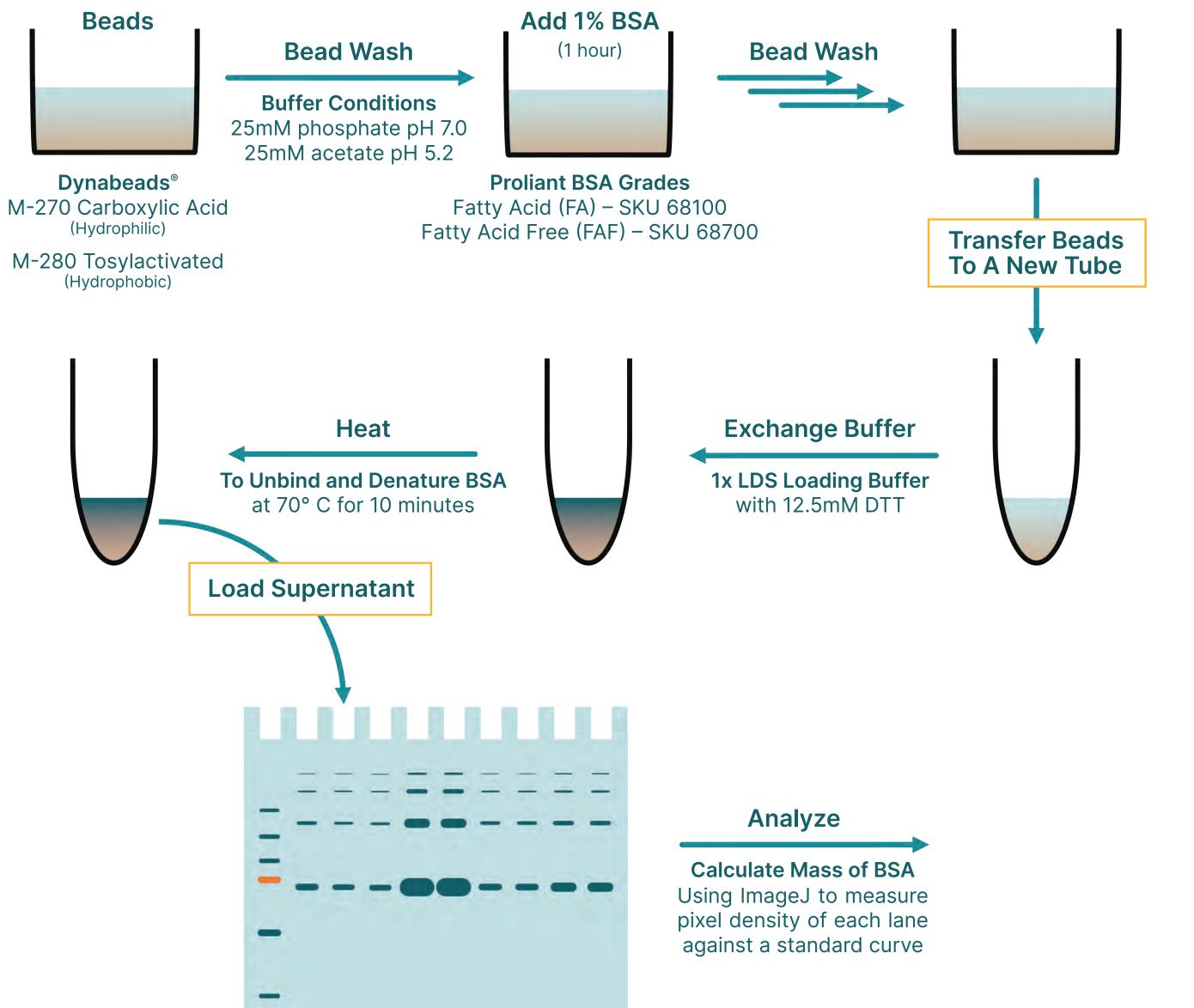


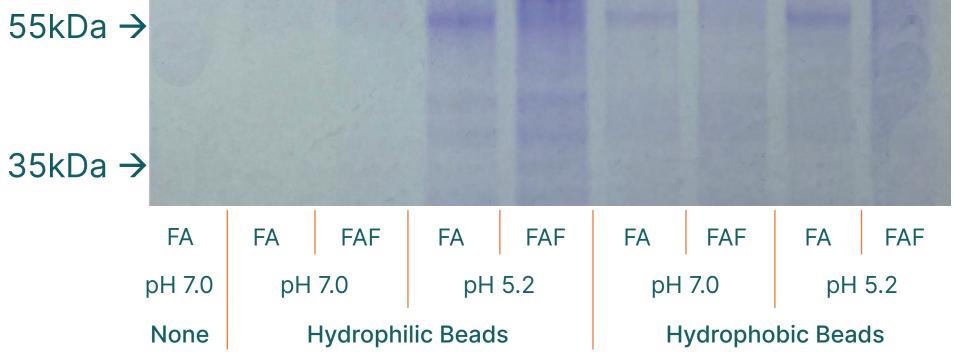
More BSA Binds to Beads at pH 5.2 than at pH 7.0



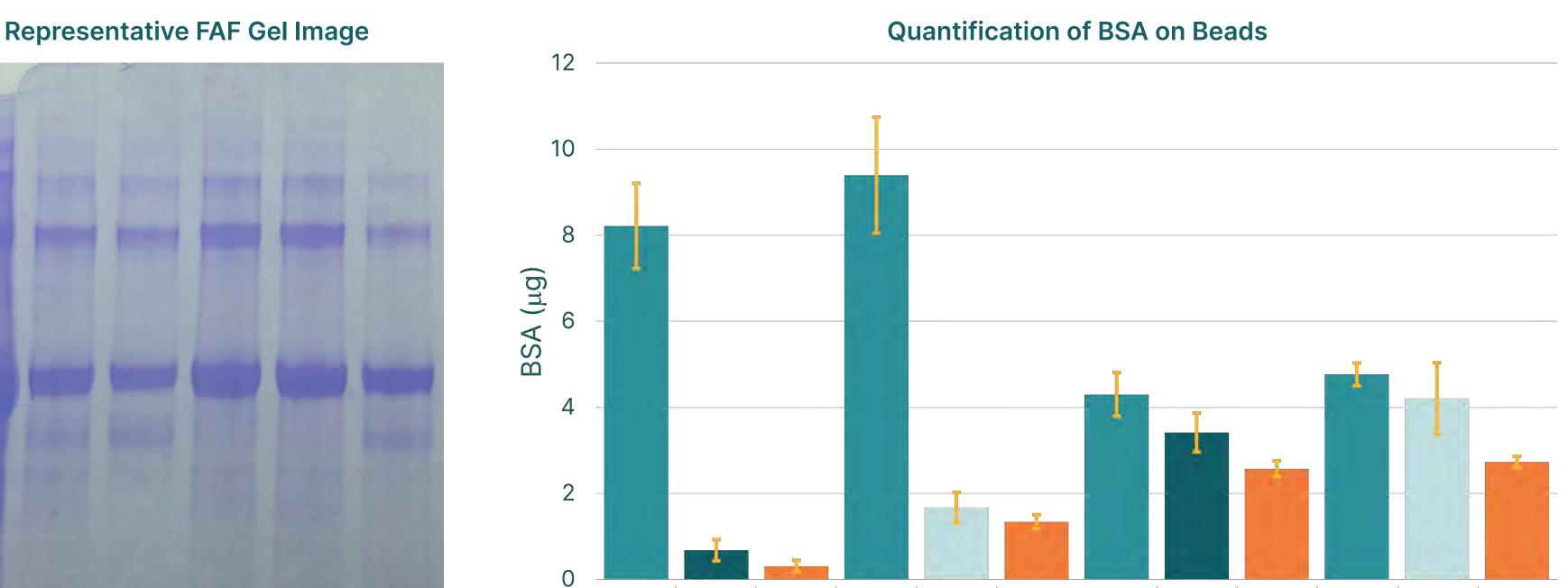
Methods

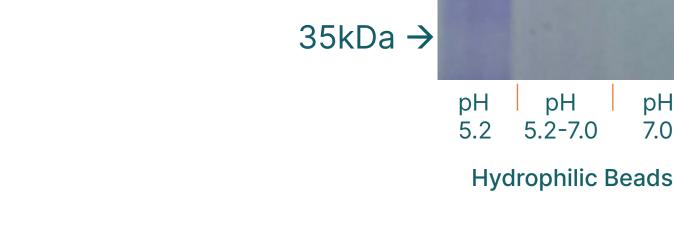
BSA Binding





The Binding at pH 5.2 is Reduced When the Beads Are Washed at pH 7.0





250kDa →

130kDa →

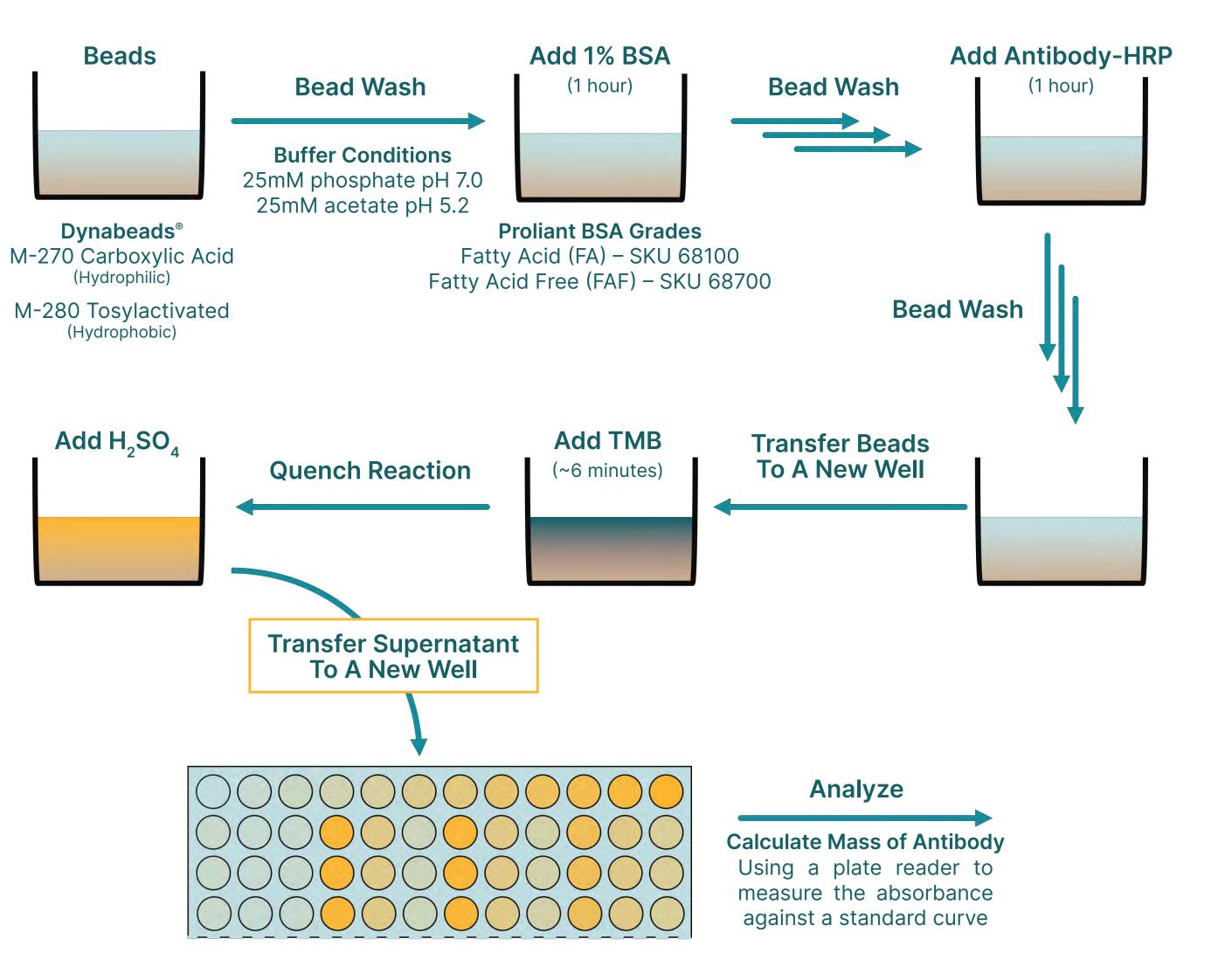
100kDa →

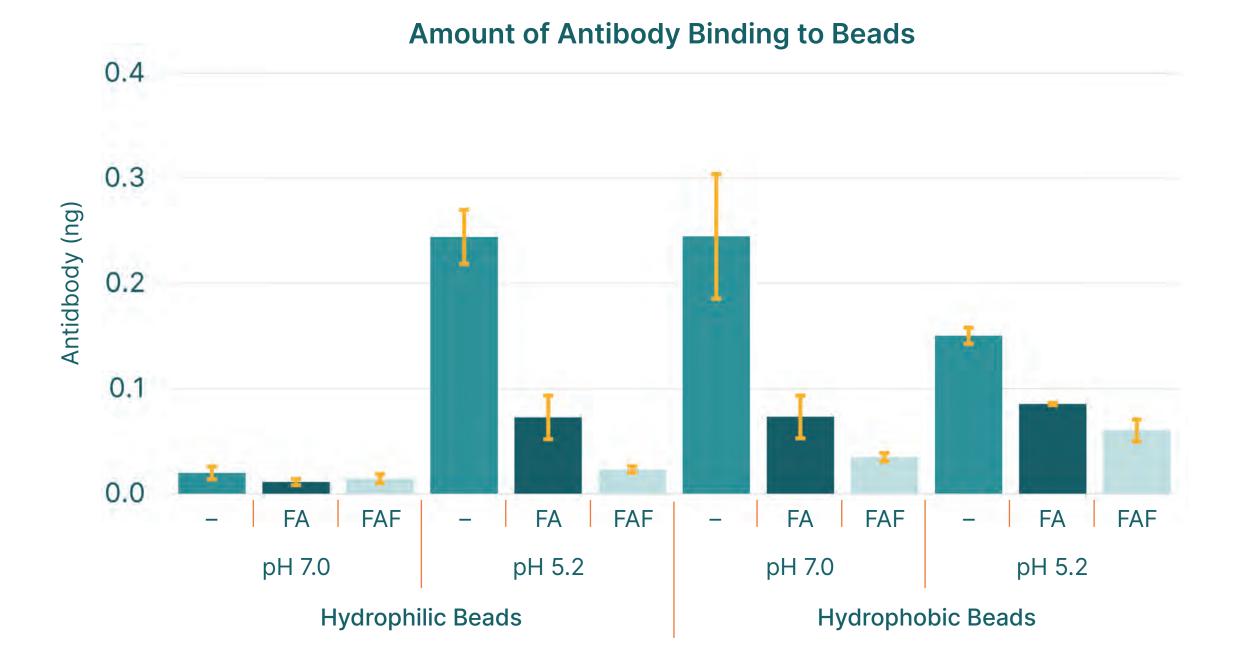
70kDa →

55kDa →

| pH pH pH
5.2 5.2-7.0 7.0 |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Fatty Acid | Fatty Acid Free | Fatty Acid | Fatty Acid Free |
| Hydrophilic Beads | | Hydrophobic Beads | |

BSA Blocks Nonspecific Antibody-Bead Interactions in Every Tested Condition





рΗ

Hydrophobic Beads

5.2 5.2-7.0

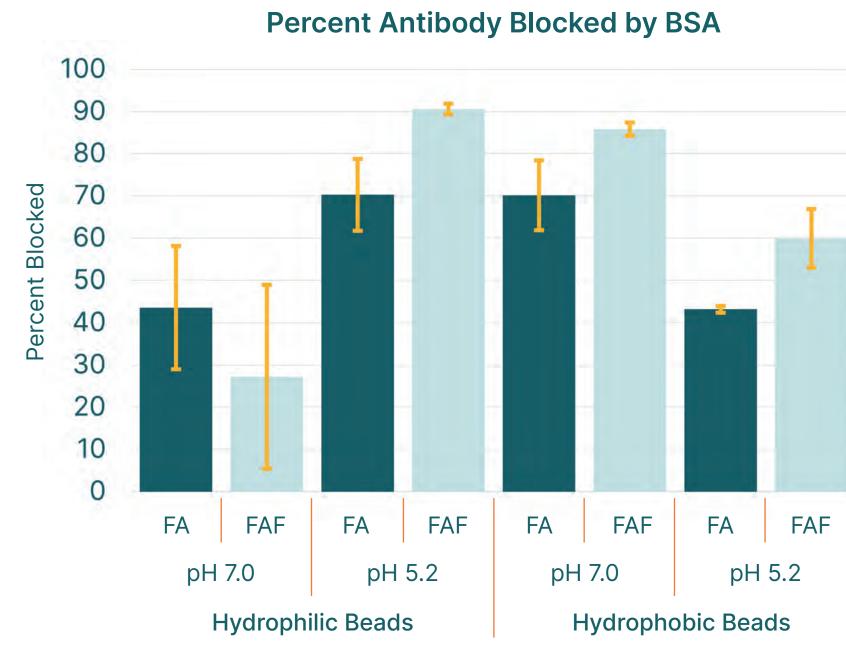
рΗ

pН

7.0

pН

7.0



Conclusions

• The presence of BSA reduces the noise within bead-based ELISAs by binding to the bead and blocking nonspecific

BSA Blocking

*Error bars show the standard deviation

antibody-bead interactions, regardless of BSA grade, bead type, and pH.

• FAF grade BSA demonstrates slightly better blocking than FA grade BSA, but both FA and FAF BSA decrease nonspecific antibody-bead interactions.

• BSA shows antifouling properties in all tested conditions, but lowering to pH 5.2 significantly increases BSA bead coating.

• The coating of the BSA at pH 5.2 does not remain high when washed with a pH 7 buffer, especially on hydrophilic beads.

• Further studies using QCM-D and AFM will be used to understand binding kinetics and energetics, surface coverage uniformity, BSA deformation, and coating thickness under the different conditions including BSA grade, bead type, and pH.