StabilZyme SELECT®

Stabilizer

Recommendations for Use

StabilZyme *SELECT*[®] Stabilizer, Product No. SZ03, is effective for HRP conjugates, antibody-coated particles, and unmodified proteins in solution. Use in all types of assays: competitive, direct, or sandwich.

This product is passed through a 0.1 micron filter and contains a mercury-free, azide-free preservative.

- 1. Use StabilZyme *SELECT* stabilizer straight or dilute 1:1 in deionized water or 0.1 M Tris or Glycine buffer*. (Phosphate buffers cause precipitation at >2mM concentration in the final volume.)
- 2. Adjust pH to 6.8 7.0.
- 3. Dilute your reagent to optimum concentration in the StabilZyme *SELECT* stabilizer solution.
- 4. Use diluted reagent in your assay as normal.
- 5. Store the conjugate solution at 4°C. Protect from direct exposure to light.

*StabilZyme *SELECT* Stabilizer has limited buffering capacity. In competitive assays, add buffer if the test samples have extreme pH variability.

Technical Assistance

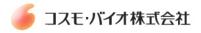
For technical inquires, please call Pamela Reed at 952-278-2638 or email preed@surmodics.com.

Ordering Information

StabilZyme SELECT Stabilizer is available in these sizes:			
125 mL	1000 mL	2000 mL	
SZ03-0125	SZ03-1000	SZ03-2000	

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StabilZyme SELECT®

Stabilizer

StabilZyme *SELECT*[®] Stabilizer stabilizes biologically active components in solution and incorporates a unique technology to protect the activity of proteins commonly used in diagnostic immunoassays, such as antisera controls and Horseradish Peroxidase conjugates.

Consistent.

By storing your HRP-hapten conjugate in StabilZyme *SELECT* stabilizer, the standard curve of your assays can be maintained over time, improving the accuracy and consistency of your tests.

Quality.

StabilZyme *SELECT* stabilizer is manufactured per ISO 9001 and 13485 standards under stringent controls to ensure lot-to-lot consistency and traceability.

Time.

StabilZyme *SELECT* stabilizer is ready-to-use, eliminating the preparation time of producing your own biomolecule stabilizer.





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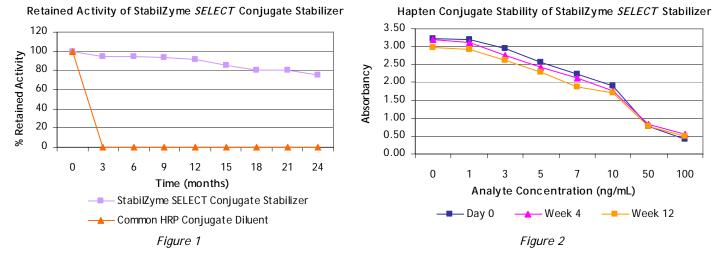
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Stability Data

Real-time stability studies conducted on monoclonal antibody-HRP conjugates that included independent testing of the enzyme activity and the immunological activity by ELISA indicated superior performance of StabilZyme *SELECT* stabilizer compared to a common HRP conjugate buffer (Figure 1). StabilZyme *SELECT* stabilizer retained over 75% of its activity after 24 months at room temperature.

In an accelerated stability study using a competitive ELISA for a drug, hapten-HRP conjugate was diluted in StabilZyme *SELECT* stabilizer to use concentration and then stored at 37°C. To test the conjugate activity, the target analyte was diluted in urine at concentrations ranging from 0-100 ng/ml, and then mixed with equal parts of the conjugate solution and applied to antibody-coated microtiter plates. Results show that the standard curve remained virtually the same after 12 weeks at 37°C. This contrasts to the complete loss of activity when the conjugate was stored in typical conjugate diluent. These results indicate a real-time shelf-life of the conjugate stored in StabilZyme *SELECT* stabilizer of two years at 4°C.



Properties & Applications

- StabilZyme SELECT stabilizer is easy to evaluate and is readily incorporated into virtually any assay production protocol. Conjugates can be added directly to StabilZyme SELECT stabilizer, and are then ready for storage and use.
- No additional components are required before including StabilZyme SELECT stabilizer into immunoassay formats. Compared to typical immunoassay diluents, background is often reduced, and binding is increased, resulting in an increased signal-to-noise ratio. For optimum efficacy, the final solution should contain at least 50% StabilZyme SELECT stabilizer.
- StabilZyme *SELECT* stabilizer is highly recommended for HRP-hapten conjugates used in competitive assays. It is also recommended for protein, serum, and urine controls and proteins conjugated to fluorescent enzymes, such as FITC.
- StabilZyme *SELECT* stabilizer is produced and aseptically filtered in accordance with an ISO-certified quality system. Extensive quality controls ensure lot-to-lot consistency and product quality. StabilZyme *SELECT* stabilizer contains a mercury-free, azide-free preservative and is a proven critical component of several commercially available devices.
- More than 1.5 mM phosphate or 10% phosphate buffered saline (PBS) in StabilZyme *SELECT* stabilizer will cause precipitation. To prevent this, use normal saline (0.85% NaCl) rather than a phosphate buffer. StabilZyme *SELECT* stabilizer will not improve the stability of the bond between the enzyme and the antibody or antigen.



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Protein Stability Solutions: An Overview of Products for *In Vitro* Applications

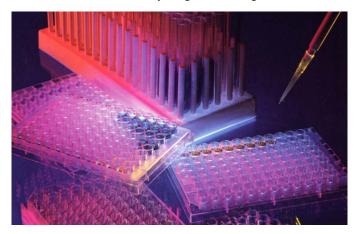
SurModics Inc., a publicly traded company on NASDAQ (symbol: SRDX), is a leading provider of surface modification and drug delivery solutions for medical device and biomedical applications, as well as proprietary in vitro diagnostics products to improve the performance, stability, and immobilization of biomolecules. SurModics has been developing protein stabilization products since the introduction of its StabilCoat[®] Immunoassay Stabilizer in 1990. Since that time, SurModics has introduced several other products for the stabilization of proteins, both dried and in solution. These products have shown unsurpassed stabilizing efficacy when compared to leading competitor products. Through its state-of-the-art technologies and products, SurModics brings innovation together with biomedical companies around the world.

PROTEIN STRUCTURE AND STABILITY

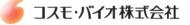
Proteins, comprised of amino acid chains, are flexible polymeric molecules with functional three-dimensional catalytic or binding sites [1]. The spatial arrangement of amino acids in the catalytic and/or binding site is depend-



ent upon the interaction of these amino acids with one another, the solvent and solute molecules [2-4]. It is generally believed that structure of proteins, along with environmental conditions, determine protein stability. There are four levels of protein structure, i.e., primary, secondary, tertiary, and quaternary. The primary structure of a protein is the linear sequence of its constituent amino acids. As this linear structure begins to fold, it forms a secondary structure. The two most common secondary structures are the alpha helix and the beta sheet. The alpha helix is a tightly coiled polypeptide chain and is found in many different types of proteins in different amounts and configurations. Intramolecular hydrogen bonding between amino



hydrogen groups and carbonyl groups stabilizes this structure. The beta sheet is similar to the alpha helix in that it uses extensive hydrogen bonding to stabilize itself but it is completely different in structure. The polypeptide chains of the beta sheet are almost completely extended and the hydrogen bonds are found between different polypeptide chains instead of within the same chain like the helix. Tertiary structures of proteins are formed as secondary structures become farther apart along the polypeptide chain. As these polypeptide chains begin to interact with their respective side chains, this more complex level of folding is created. Some proteins are composed of more than one polypeptide. Each polypeptide is called a subunit. The quaternary structure of proteins results from the interaction of the polypeptides of a multi-subunit protein-in other words, it is the spatial arrangement of subunits within a protein [5].



Proteins comprise both hydrophilic and hydrophobic amino acids. Therefore, their composition results in spontaneous folding in aqueous solution – more hydrophobic amino acids are clustered in the interior of the globular molecule whereas the polar amino acids are concentrated in the hydrated exterior [6,7]. In addition to hydrophobic interactions, the electrostatic interactions, hydrogen bonding and van der Waals are major forces involved in the folding process. Proper folding ensures biological activity of the protein. Proteins in the native state are folded and are most stable [8,9]. However, once a protein is folded, it is not invincible. External factors such as temperature, pH, and the presence of salts, metal ions or other contaminants may denature a protein. Proteins may also denature when adsorbed to the side of a laboratory vessel. Therefore, proteins have to be stored and used in an environment conducive to stabilizing their native structure. Proteins are used and stored in dry form as well as in solution. However, drying and freezing processes can result in denaturation of proteins if proper stabilization conditions are not utilized at crucial stages of preparation, storage, and handling. Regarding storage, proteins in solution generally have a shorter shelf-life than dried forms.

PROTEIN STABILIZATION REAGENTS

Proteins are heavily used in numerous industrial and research applications, such as pharmaceutical, diagnostic and therapeutic agents. Diagnostic immunoassays, such as enzyme linked immunosorbent assay (ELISA), are important and sensitive analytical methods that are widely used for numerous environmental, genetic, clinical, and biochemical studies [10,11]. These assays utilize proteins (e.g., antigens and antibodies) immobilized to a solid support along with detection proteins labeled with chromogenic or fluorescent molecules, or conjugated with other biomolecules such as horseradish peroxidase, alkaline phosphatase or beta galactosidase.

A variety of commercially available protein stabilization reagents are currently on the market. Scientists are continually researching ways to create products for the stabilization and storage of a variety of biologicals to provide an environment close to their native conditions. Different types of proteins, enzymes and cells need proper strategies for handling and storage to maintain maximum functionality for therapeutic and diagnostic applications. However, the best conditions for one biomolecule may not be the same for another. Therefore, multiple solutions need to be designed and applied to ensure stability.

SurModics has developed several products for maintaining the stability of proteins. These products are designed to preserve the conformation of proteins in their native folded state when dried, such as in coated plates or membranes, or in solution, such as enzyme conjugates or serum controls. Additional products are designed for enhancing the performance of multiplexed assays such as microspheres and protein microarrays. Some of the products are animal protein-free to avoid any interactions with test proteins.

SurModics' stabilization products are produced and aseptically filtered in accordance with an ISO-certified quality system. Extensive quality controls ensure lot-to-lot consistency and product quality. These products are proven critical components of many commercially available diagnostic devices and test kits. The StabilZyme[®] stabilizers, as well as StabilCoat[®] Plus and StabilGuard[®] Choice stabilizers contain a mercury-free, azide-free preservative.

STABILIZATION OF DRIED PROTEINS

While most specific-binding proteins function in the aqueous state in nature, the dry or frozen state is much preferred for stable storage. In these relatively immobile states, the frequency of collision with harmful co-solutes, such as proteases and oxidants, is immensely reduced. However, removal of solvent from protein molecules through drying – or through other phase changes such as precipitation and freezing – puts stress on the functional conformation of proteins. These phase changes can expose the hydrophobic amino acids normally buried within the molecule. Such exposure increases the proteins' association with other molecules and with hydrophobic surfaces, resulting in denaturation of the protein molecules.

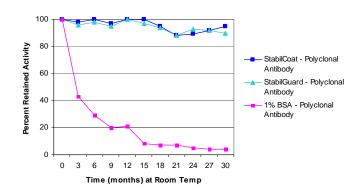
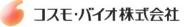


Figure 1. Polyclonal antibody stability with StabilCoat Immunoassay Stabilizer and StabilGuard Biomolecule Stabilizer. For 30 months, polyclonal and monoclonal (see Figure 2 below) antibodies were stabilized with either StabilCoat Immunoassay Stabilizer, StabilGuard Biomolecule Stabilizer or 1% BSA in microtiter plates and placed at room temperature. The percent retained activity was determined by comparing the activity of the aged antibody-coated microtiter plates to that of a freshly coated plate.

Both drying methods and storage conditions are critical to maintaining stability of proteins. Membrane applications generally require very fast drying or lyophilization to retain optimum performance and activity. All dried products should be stored in an airtight container with a desiccant for maximum shelf life [12].

Inclusion of compatible solutes in the drying solution to prevent denaturation is an effective approach to stabilizing proteins for storage. These solutes typically contain



hydrophilic groups which stabilize the functional conformation of the protein molecules by burying the hydrophobic amino acids within. Therefore, it is important to apply an appropriate stabilizing solution before drying to prevent protein denaturation.

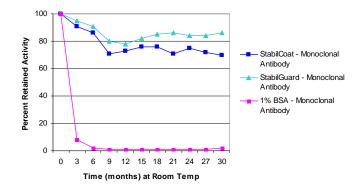


Figure 2. Monoclonal antibody stability with StabilCoat Immunoassay Stabilizer and StabilGuard Biomolecule Stabilizer (see description in Figure 1 caption above).

SurModics has developed two products to maintain the activity of dried antibodies and other proteins adsorbed or immobilized on solid substrates: StabilCoat Immunoassay Stabilizer and StabilGuard Biomolecule Stabilizer (see descriptions below and Figures 1 & 2). StabilGuard stabilizer is completely synthetic and does not contain any protein. Independent research studies and studies done inhouse, have proven superior performance of these products over other commercial competitive products [10,13].

STABILCOAT[®] IMMUNOASSAY STABILIZER

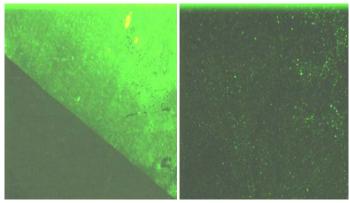
StabilCoat Immunoassay Stabilizer effectively preserves the conformation and activity of dried protein components in immunoassays. Using StabilCoat stabilizer as a coating or lyophilization buffer protects antibodies, enzymes, and antigens from the denaturing effects of drying. StabilCoat also keeps assays at peak performance over time by protecting against the detrimental effects of elevated temperatures and surface interactions.

STABILGUARD[®] BIOMOLECULE STABILIZER

StabilGuard Biomolecule Stabilizer maintains the activity of antibodies and other biomolecules that are adsorbed or immobilized to solid substrates. Unique chemistries allow this solution to both stabilize biomolecule activity and block nonspecific binding sites without incorporating animal protein. This virtually eliminates the possibility of test interference or cross reactivity that can result from the use of animal protein. As a blocking agent, StabilGuard stabilizer reduces background noise when compared to other typical blocking agents. Minimal incubation is required for effective blocking (see Figure 3).

STABILIZATION OF PROTEINS IN SOLUTION

Proteins in solution are more susceptible to temperature fluctuations, contamination with oxidative and microbial agents, as well as physical shearing and shaking. As a general rule, proteins in solution are the most stable at low temperatures and high concentrations. At temperatures just above freezing, proteins have less of the kinetic energy required to 'escape' their minimum energy functional conformation(s). At higher protein concentrations (mg/ mL range), a lower fraction of the total protein activity is lost through the adsorption of the protein molecules to the container surface, the dissociation of multiunit enzymes, and inactivation by low levels of contaminants.



A. Untreated Surface (Control Buffer) B. Surface Treated with StabilGuard

Figure 3. Reduction in nonspecific binding with StabilGuard Biomolecule Stabilizer. The surfaces of metallic samples were inoculated with mouse IgG diluted in a control buffer (A) or 1:2 in StabilGuard stabilizer (B), placed in 6-well culture plates and dried at 37°C. After rinsing to remove unbound antibody, goat anti-mouse IgG FITC solution was added to the wells and plates were agitated at 37°C for one hour. Images were captured using a fluorescent microscope. The images above show that the mouse IgG bound nonspecifically to the surface when a control buffer was used; however, the sample prepared with StabilGuard stabilizer shows a dramatic reduction in nonspecific binding of the primary antibody.

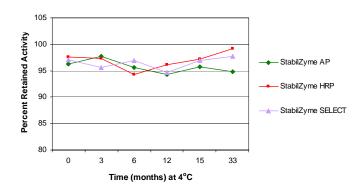


Figure 4. Shelf Life of StabilZyme Conjugate Stabilizers. Real-time studies were used to evaluate the shelf life of StabilZyme HRP, AP and SELECT Conjugate Stabilizers. The StabilZyme Conjugate Stabilizers were stored at 4°C and tested at various time points over 33 months. A monoclonal antibody conjugate (alkaline phosphatase for StabilZyme HRP and SELECT Conjugate Stabilizers) was diluted to use concentration and then stored in the corresponding StabilZyme Conjugate Stabilizer. The enzyme activity was tested by ELISA. The percent activity was determined by comparing the activity of the stored StabilZyme Conjugate Stabilizer to that of a fresh lot of StabilZyme Conjugate Stabilizer.



Protein source, purification method, and conjugation chemistry may affect the stability, and therefore, the activity of proteins. For example, the source of an enzyme, such as alkaline phosphatase, may have a profound effect on conjugate stability. In addition, it is essential that any proteolytic enzymes present in the protein source, e.g., hydrolases and oxidases, are removed or inactivated.

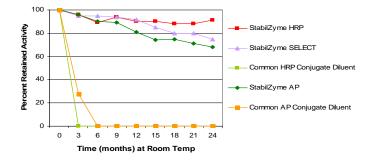


Figure 5. Stability of HRP conjugated monoclonal antibody stored in StabilZyme HRP Conjugate Stabilizer, StabilZyme *SELECT* Conjugate Stabilizer, or a common HRP conjugate diluent, and stability of AP conjugated to monoclonal antibody stored in StabilZyme AP Conjugate Stabilizer or a common AP conjugate diluent. The conjugated monoclonal antibodies were diluted to 0.4 µg/mL in each of the stabilizers and then stored at room temperature for 24 months. Activity was tested by ELISA.

Different proteins may be conjugated to enzymes for detection of biomolecules in solution. SurModics has developed several protein stabilizers for specific enzymeantibody conjugates. These stabilizers can be used as diluents to store conjugated proteins at lower use concentrations.

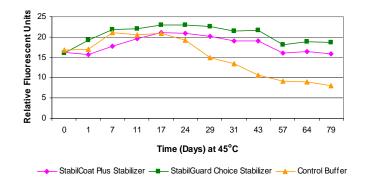


Figure 6. Enhanced stability of polyclonal antibodies. Rabbit polyclonal antibodies were attached to microspheres through standard EDC coupling and stored in StabilCoat Plus stabilizer, StabilGuard Choice stabilizer or a control buffer at 45°C. Both StabilCoat Plus and StabilGuard Choice stabilizers retained >96% binding activity after 79 days, corresponding to >1 year at room temperature.

STABILZYME[®] HRP CONJUGATE STABILIZER

StabilZyme HRP Conjugate Stabilizer was developed specifically for horseradish peroxidase (HRP) conjugates in solution. Its components are a unique combination of proprietary ingredients designed to protect the entire conjugate. StabilZyme HRP stabilizer simultaneously prevents the loss of catalytic activity while maintaining the conformation of the antibody or protein antigen portion of the conjugate. StabilZyme HRP stabilizer allows conjugate storage at use concentration, resulting in stable conjugates that require no dilution. It is an excellent diluent, and often decreases background and increases signal for improved signal-to-noise ratios (see Figures 4 & 5).

STABILZYME[®] AP CONJUGATE STABILIZER

StabilZyme AP Conjugate Stabilizer was developed specifically for alkaline phosphatase (AP) conjugates in solution. It is a unique combination of proprietary ingredients that prevents the loss of catalytic activity while maintaining the stability of the proteins in solution. StabilZyme AP stabilizer allows conjugate storage at use concentration, resulting in stable conjugates that require no dilution. Like StabilZyme HRP stabilizer, it is an excellent diluent and often decreases background and increases signal for improved signal-to-noise ratios (See Figure 4).

STABILZYME SELECT® STABILIZER

StabilZyme SELECT Stabilizer is a diluent and stabilizer for biologically active components in solution. It incorporates a unique technology that protects the activity of proteins commonly used in diagnostic immunoassays, such as antisera controls and horseradish peroxidase conjugates. No additional components are required when using Stabil-Zyme SELECT stabilizer in immunoassay formats. Compared to typical immunoassay diluents, background is often reduced, and binding is increased, resulting in an increased signal-to-noise ratio. StabilZyme SELECT stabilizer is recommended for protein, serum, and urine controls, proteins conjugated to fluorescent enzymes, and HRP-hapten conjugates used in competitive assays. By storing a HRP-hapten conjugate in StabilZyme SELECT stabilizer, the standard curve of assays can be maintained over time, improving the accuracy and consistency of tests (see Figures 4 & 5).

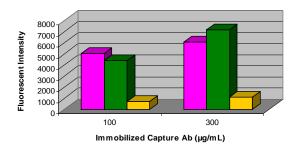
STABILIZATION OF PROTEINS BOUND TO MICRO-SPHERES OR MICROARRAYS

To achieve high throughput, multiplex assays, such as microarrays and multiplex ELISA, have been designed. These methods employ the use of microspheres or glass slides with multiple antigens or antibodies. Biomolecules captured by the bound proteins are detected by different methods, such as flow cytometry or laser scanning. Stabilizing solutions for proteins bound to microspheres or slides can stabilize the proteins, and also block nonspecific binding, resulting in improved sensitivity of the system. SurModics has developed two products for this purpose, StabilCoat[®] Plus Stabilizer and StabilGuard[®] Choice Stabilizer. StabilGuard Choice stabilizer is completely synthetic and does not contain any animal protein.



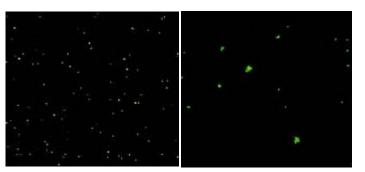
STABILCOAT[®] PLUS STABILIZER AND STABILGUARD[®] CHOICE STABILIZER

StabilCoat Plus Stabilizer and StabilGuard Choice Stabilizer improve the stability of proteins bound to microspheres in solution, protect the activity of proteins while blocking nonspecific binding sites, and prevent aggregation even after long-term storage. For microarrays, they improve protein array performance by increasing signal, reducing background, improving sensitivity, and effectively preserving conformation and activity of array proteins stored long-term in the dried state (Figures 6, 7 & 8).



StabilCoat Plus Stabilizer StabilGuard Choice Stabilizer Control Buffer

Figure 7. Enhanced signal intensity in antibody microarrays. Monoclonal antibody to IFN γ (100 and 300 µg/mL) was arrayed on a SurModics proteinbinding surface using BioRobotics split pins and a BioRobotics arrayer. Cocktail antigens containing recombinant human IFN γ at 25 ng/mL were incubated on slides using a control buffer, StabilCoat Plus Stabilizer or StabilGuard Choice Stabilizer. After incubation, arrays were developed with biotinylated antibody cocktail suspended in the same reagents, respectively. The spots were visualized by incubating with Streptavidin Cy5.



A. Microspheres stored in StabilCoat Plus Stabilizer

B. Microspheres stored in control buffer

Figure 8. The colloidal stability of StabilCoat Plus Stabilizer versus a control buffer. Studies were conducted to evaluate the effectiveness of StabilCoat Plus Stabilizer and StabilGuard Choice Stabilizer to maintain colloidal stability of polystyrene microspheres in solution. Polystyrene microspheres with rabbit polyclonal antibody covalently attached were stored in either Stabil-Coat Plus Stabilizer, StabilGuard Choice Stabilizer or a control buffer. Both products demonstrated the ability to prevent aggregation. Fluorescent images of microspheres stored in StabilCoat Plus Stabilizer (A) and a control buffer (B) are shown above.

CONCLUSION AND FUTURE PLANS

Proteins must be stored and handled in ways that will provide stability and viability for as long as possible. Proteins are complex molecules, thus their stability issues are complex. The best conditions for stabilizing one protein may not be the same for another.

SurModics is committed to enriching its existing stabilization products by developing new products to meet the ever-changing needs of researchers in academia and industry — our scientists are continually working to develop new products for protein storage and use in an environment that closely resembles their native conditions.

In 2005, SurModics will introduce a BSA-free (bovine serum albumin-free) conjugate stabilizer to its StabilZyme product line. This product virtually eliminates the possibility of test interference or cross-reactivity in solution that can result from the use of bovine protein.

SurModics is also exploring the possibility of stabilization/ storage products beyond proteins; this includes biologicals, such as stem cells, that are anticipated for use in a variety of therapies.

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Table 1. SurModics Stabilization Products: Applications and Properties

Product Name	Applications	Properties
StabilCoat [®] Immunoassay Stabilizer	 For Dry Stabilization - Preserves the conformation and activity of dried components in immunoassays Can be used to stabilize antibodies, enzymes, and antigens on an assortment of immunoassay compo- nents, such as polystyrene plates, tubes, glass, membranes and filter paper Excellent for bulk stabilization of lyophilized antibod- ies, enzymes, antigens, etc., to protect against dena- turing effects of drying 	 pH 7.0 – 7.4 Contains purified bovine protein Does not contain a preservative 5X concentrate also available
StabilGuard [®] Biomolecule Stabilizer	 For Dry Stabilization - Preserves the conformation and activity of dried components in immunoassays Stabilizes activity and blocks nonspecific binding sites at the same time Can be used as a blocking agent and/or stabilizer for antibodies, enzymes, and antigens on an assortment of immunoassay components, such as polystyrene plates, tubes, glass, membranes and filter paper Not recommended as a bulk stabilizer for freeze- dried antibodies, etc. 	 pH 6.6 - 7.2 StabilGuard is made with all synthetic materials (No protein or animal derived components) Does not contain a preservative Minimal incubation time required for blocking
StabilZyme [®] HRP Conjugate Stabilizer	 For Liquid Stabilization Developed specifically for HRP conjugates in solution; prevents loss of catalytic activity of the HRP and maintains the conformation of the antibody or antigen portion Allows conjugate storage at use concentration 	 pH 6.2 - 6.7 Contains purified bovine protein Preserved with 0.02% methyl- isothiazolone, 0.02% bromoni- trodioxane, and 20 ppm Proclin 300
StabilZyme [®] AP Conjugate Stabilizer	 For Liquid Stabilization Developed specifically for alkaline phosphatase (AP) conjugates in solution; prevents loss of catalytic activity of the AP and maintains the conformation of the antibody or antigen portion Allows conjugate storage at use concentration 	 pH 5.5 – 6.5 Contains purified bovine protein Preserved with 0.02% methyl- isothiazolone and 0.02% bro- monitrodioxane
StabilZyme <i>SELECT[®]</i> Conjugate Stabilizer	 For Liquid Stabilization Diluent and stabilizer for biologically active components in solution Recommended for: Protein controls (especially antibodies) HRP-hapten conjugates used in competitive assays Applications where background is a problem 	 pH 6.5 – 7.5 Contains purified bovine protein Preserved with 0.02% methyl- isothiazolone and 0.02% bro- monitrodioxane
StabilCoat [®] Plus Stabilizer and StabilGuard [®] Choice Stabilizer	 For Microsphere and Microarray Applications These products improve the stability of proteins bound to microspheres in solution, protect the activity of proteins while blocking nonspecific binding sites, and prevent aggregation These products improve protein array performance by increasing signal, reducing background and improving sensitivity, and effectively preserving the conformation and activity of array proteins stored long-term in a dried state 	 StabilCoat Plus, pH 7.0 – 7.4 StabilGuard Choice, pH 6.6 – 7.2 StabilCoat Plus contains purified bovine protein StabilGuard Choice is made with all synthetic materials (No protein or animal derived components) Both products are preserved with 0.02% methylisothiazolone and 0.02% bromonitrodioxane