Overview of Stabil™ Family of Immunodiagnostic Reagents

University of Wisconsin
Madison, WI
August 19, 2010
SurModics Overview

- Established 1979
- Public offering 1998 (SRDX)
- 250 employees
- Over 100 licensed customers
- $121.5M in revenue (FY09)
- Profitable; strong balance sheet
- 1 goal: to get our partners’ products to market

Eden Prairie, MN
- Corporate headquarters
- Drug delivery, surface modification and in vitro technologies

Birmingham, AL
- Pharmaceutical division
- Microparticle and implant drug delivery platforms
- cGMP manufacturing facilities

Improving patient lives through technology innovation
Industry Solutions

Medical Devices
Coating of electrical and mechanical instruments
• Enable drug delivery from device to reduce infections, enhance cell growth, reduce inflammation
• Coat devices to make more biocompatible
• Coat devices for easier maneuverability and faster surgical implantation
• Develop fully biodegradable devices for tissue regeneration, wound healing, delivery of therapeutic agents

Pharmaceuticals
Parenteral drug delivery
• Local and systemic drug delivery
• Custom drug formulation to allow delivery from weeks to months
• Provide local delivery where systemic is not needed or contra-indicated
• Enable ability to lower dose, reduce injection frequency, and offer alternative modes of drug administration

Life Sciences
In vitro diagnostic test components and surfaces
• Diagnostic assay components and technologies
• Surface chemistry products and coatings for biological attachment
• Microarray slides
In Vitro Diagnostics

SurModics provides the world’s foremost innovative in vitro diagnostic assay components and technologies enabling the world’s leading and emerging diagnostic, medical device, pharmaceutical, and life science companies to create exceptional products and systems

- Protein stabilization reagents
- Blocking buffers
- BioFX® colorimetric and chemiluminescent substrates
- CodeLink® microarray slides
- TRIDIA™ diagnostic surfaces
- DIARECT autoimmune antigens
- BioFX secondary antibodies
- BioFX blocks, diluents, wash solutions, and stop reagents
Stabilization Products for \textit{In Vitro} Diagnostics

Gold Standard in Protein Stabilization and Surface Passivation (Blocking)

Stabilizers and Blockers for a variety of applications

- Coated surfaces
- AP and HRP conjugates
- Proteins in solution
- Microarrays
- Heterophilic antibody blockers
- Blotting blockers
- Microfluidic blockers
- Lateral flow
Overview - What is a stabilizer?

- Stabilizers are reagents that have been designed to preserve the conformational spatial arrangement of proteins in their native folded state when dried or in solution.

- Keep hydrophobic regions of the protein buried inside itself.

- Proper protein folding ensures the biological activity of the protein.

- External factors that affect protein-folding:
  - Temperature
  - pH
  - salts
  - metal ions
  - other contaminants
Stabilization Product Lines

• **StabilCoat® Stabilizer**  
  – Stabilize proteins coated to a surface

• **StabilGuard® Stabilizer**  
  – Synthetic protein stabilizers

• **StabilZyme® Stabilizer**  
  – Stabilize proteins – in solution
StabilCoat® Stabilizer – Dried Stability Study

- After 30 months at room temperature
  - Rabbit monoclonal antibody maintained 70% of its activity
  - Rabbit polyclonal maintained 95% of its activity

\[ \% \text{Retained activity} = \frac{\text{Average OD } @ \ 37^\circ \text{ C}}{\text{Average OD } @ \ 4^\circ \text{ C}} \times 100 \]
• **SurModics StabilGuard** and **StabilCoat** Stabilizers as Blockers

1. **Microtiter plate**

2. **Blocker: StabilCoat or StabilGuard Stabilizer**
In-House Stability Test Method

- Troponin sandwich ELISA
- Coat a mouse anti-Troponin capture antibody on the plate
- Stabilize with each stabilizer to be tested
- Dry down and store plates at low humidity:
  - Store at 4°C (control)
  - Store at 37°C (accelerated)
- Test 4°C and 37°C plates over 1 year
- Determine %Retained activity = \( \frac{\text{Average OD @ 37°C} \times 100}{\text{Average OD @ 4°C}} \)
**StabilGuard and StabilCoat Stabilizers**

**Dried Stability vs. Competitors**

- At the one-year stability time point, *StabilCoat and StabilGuard* stabilizers demonstrated greater than 90% retained activity.

- The sustained functional activity suggests SurModics’ stabilizers were able to preserve the functional conformation of the dried antibody.
Standard Curve Consistency

- Data above demonstrate StabilCoat stabilizer providing consistency, stability, and reproducibility of the 4°C control curve at each time point
- All EC50 values at each time point fall within two standard deviations of the mean

<table>
<thead>
<tr>
<th>Time Point</th>
<th>EC50</th>
</tr>
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<tbody>
<tr>
<td>Day 0</td>
<td>111</td>
</tr>
<tr>
<td>Day 1</td>
<td>112</td>
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<tr>
<td>1 week</td>
<td>129</td>
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<td>3 months</td>
<td>139</td>
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<td>6 months</td>
<td>149</td>
</tr>
<tr>
<td>9 months</td>
<td>154</td>
</tr>
<tr>
<td>1 year</td>
<td>146</td>
</tr>
<tr>
<td>mean</td>
<td>133</td>
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<tr>
<td>std dev</td>
<td>16.7</td>
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<tr>
<td>%CV</td>
<td>12.6</td>
</tr>
<tr>
<td>2 SD range</td>
<td>99.6 - 166.4</td>
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</table>
**StabilGuard and StabilCoat Dried Stability vs. Common In-House Stabilizers**

- After one week at 37°C storage, *StabilCoat and StabilGuard* stabilizers demonstrated almost 100% retained activity.
- Common in-house stabilizers fall below 50% retained activity.
StabilGuard® Products: Why Synthetic?

- Synthetic or BSA-free
  - BSA in blocking solutions can affect assay performance
Heterophilic Interference Blocker, in Product Development

- **Heterophilic antibody**: All human antibodies which are found in patients' serum or plasma and bind to IgG / IgM of other species
  - HAMA are the most common
- **Problem?**
  - Produce false positives and false negatives in diagnostic immunoassays
  - Reduce efficacy of antibody based anti-therapeutics
    - Most anti-therapeutics produced in mouse monoclonal antibodies

![Diagram showing routine sandwich and heterophilic false positive](image-url)
• Article demonstrating false positive results in a Luminex based assay

• Assay format: Multiplexed indirect assay for measuring antibodies to Pneumococcal polysaccharides (PnP)s

Pneumococcal antibodies

Pneumococcal antigens

• Jerry Pickering at ARUP Laboratories (Salt Lake City, UT)

• Used with permission from the American Society for Microbiology
StabilGuard® Stabilizer in Microsphere Assays

Sample demonstrating high polyspecific reactivity to BSA

- Very high levels of nonspecific reactivity demonstrated in 33 samples
- Protocol #2: Incorporated StabilGuard stabilizer as the microsphere resuspension buffer, non-specific reactivity of the sera to the microspheres was eliminated, on all but a few sera samples
- ARUP identified the remaining samples as demonstrating polyspecific reactivity to BSA, not the microspheres
- Protocol #3: StabilGuard stabilizer as the wash buffer, the false positive reactivity of the sera to BSA was eliminated (shown in figure above)
Benefits of StabilGuard® Stabilizer in Multiplexed Luminex® xMAP Technology

• Reduces non-specific binding of human sera to microspheres

• Reduces polyspecific reactivity of human sera to BSA

• Incorporates easily into Luminex assays at multiple steps
  – Storage buffer
  – Blocking buffer
  – Wash buffer
Overall Benefits of *Stabil* Blockers

- **Minimize unwanted protein interactions**
  - Reduced false positives
- **Peace of mind**
  - Eliminate degradation concerns with shipping
  - Eliminates the need for special storage or products in inventory
- **Convenience**
  - Incorporates easily into existing protocols
- **Time-savings**
  - Block and stabilize in the same step (as little as 5 minutes)
  - Ready to use reagents (no dilution necessary)
- **Quality / Consistency**
  - Manufactured under ISO standards to ensure lot-to-lot consistency and complete lot traceability
  - For each lot, final quality control testing confirms that the lot meets pre-defined product specifications for proprietary components, physical characteristics, and performance.
StabilZyme® Products (In-Solution Stability)

- StabilZyme® HRP Conjugate Stabilizer
- StabilZyme® NOBLE Stabilizer
- StabilZyme SELECT® Stabilizer
- StabilZyme® AP Conjugate Stabilizer
StabilZyme® HRP Conjugate Stabilizer

- Stabilizes HRP conjugates in solution
  - Antibody conjugated to HRP enzyme
- Decreases background
- Increased signal for improved signal to noise ratio

After 6 months, enzymes stored in StabilZyme HRP stabilizer retained greater than 70% activity as compared to less than 10% for other competitor products
StabilZyme SELECT® Stabilizer

- Stabilizes proteins **in solution**
  - enzymes, antigens, antibodies
- Used for stabilizing **serum controls**
- Used as **diluent** for biological samples

Monoclonal IgG-HRP conjugate retained over 75% of its activity when stored for 2 years at room temperature
StabilZyme® NOBLE Stabilizer

- **BSA Free**
- Stabilizes proteins *in solution*
- Ideal for assays with BSA interferences
- Used as a diluent for biological samples

All four HRP conjugates retained at least 80% activity after 30 days at 37°C
StabilZyme® AP Conjugate Stabilizer

- Stabilizes Alkaline Phosphatase conjugates *in solution*
- Used for other phosphatase-based conjugates *in solution*

Superior stability with the StabilZyme AP Conjugate Stabilizer when compared to competitors
In-Solution Stability Journal Article (3-1-10)


- Nanobiotechnology & Bioanalysis Group (Universitat Rovira I Virgili in Tarragona, Spain)
  - Carcinoembryonic antigen (CEA): glycoprotein studied as a tumor marker
  - Amperometric biosensor assay for the detection of CEA
    - Detection limit (0.2 ng/mL)
  - Assay development/optimization:
    - Search for an in-solution antibody stabilizer
    - PBS, Trehalose, BSA and Glycerol versus StabilGuard® Stabilizer and StabilZyme SELECT® Stabilizer

- Stabilizer stability test methods:
  - Anti-CEA HRP antibodies were diluted in each of the above stabilizers
  - Each stabilizer was separated and stored at 4°C and 37°C
  - Kinetic enzyme activity assay:
    - Measured the absorbance two minutes after the addition of the stabilized conjugate to TMB
In-Solution Stability vs. Common In-House Stabilizers

- Anti-Carcinoembryonic (CEA)-HRP antibody in StabilGuard stabilizer demonstrated the greatest in-solution retained conjugate activity
  - ~85% after 222 days at 4°C
  - ~60% after 165 days at 37°C

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American Chemical Society
Introducing the Newest Family Member: StabilBlot™ Buffer for Blotting Applications
Three Steps in Western Blotting

- Western blotting combines the protein separation capabilities of PAGE (polyacrylamide gel electrophoresis) and immunohistochemistry to detect proteins from a complex matrix such as cell or tissue lysates.

1. **Step #1. SDS-PAGE**
   - Molecular Weight

2. **Step #2. Transfer**
   - Transfer

3. **Step #3. Immunoblot**
   - Detection
SurModics Western Blotting Products

- Blocking and Antibody Diluent Buffer
  - StabilBlot™ Family of Blotting Buffers – including novel protein free formulation
- Substrates – Colorimetric/Chemiluminescent
- Antibodies
- Loading Control Antibodies
- Cell Lysate Controls
Immunoblotting Considerations

• **Blocking of Membrane**
  
  – Background – “holes” allowing non-specific binding
  
  – Cross-reactivity with proteins used for blocking or antibodies used for detection
  
  – Sensitivity – eliminating interferences in antigen-antibody binding to increase signal

• **Antibody Dilution**
  
  • Cross-reactivity – effective antibody titer
StabilBlot™ Buffer – Protein Free

- Enhances sensitivity
- Prevents cross-reactivity (BSA / protein-free)
- Compatible with biotin / avidin systems
- Antibody diluent
- Use with nitrocellulose or PVDF membranes
- Compatible with either HRP or AP chemiluminescent detection
StabilBlot™ Buffer – Protein Free

Cell Lysates – GAPDH Detection

<table>
<thead>
<tr>
<th>Lysate (ugs)</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.125</th>
<th>0.063</th>
<th>0.031</th>
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<tr>
<td>StabilBlot Buffer</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>Competitor</td>
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<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Increased Sensitivity over Competitor
StabilBlot™ Buffer – Protein Free

Recombinant mouse IL-2

Increased Sensitivity and Eliminated Cross-reactivity (BSA)
StabilBlot™ Buffer – Protein Free

Human Cardiac Troponin I

Increased Sensitivity over Competitor
Increased sensitivity!

*StabilBlot* buffer shows significant improvement of detection limits over standard 5% milk buffer even with less sensitive HRP substrate.
Comparison of Chemiluminescence and Colorimetric Detection for GAPDH using StabilBlot™ Buffer

Detection with SurModics’ BioFX® ESPM Colorimetric Substrate is Comparable to the More Sensitive Chemiluminescent Substrates!
Sensitivity of Colorimetric HRP Membrane Substrates

32 ng
16 ng
8 ng
4 ng
2 ng
1 ng
0.5 ng

DABM    ESPM
Comparison of Signal Duration

- Greater substrate sensitivity
- Greater signal duration
- Better signal to noise ratio
Alkaline Phosphatase Substrates

• AP Chemiluminescent Substrates – membrane and microwell applications

  Less Sensitive

  » Super Sensitive – 450 nm (APS4)
  » Super Sensitive – 540 nm (APS5)
  » Ultra Sensitive – 450 nm (APU4)
  » Ultra Sensitive – 540 nm (APU5)

  More Sensitive

• AP Colorimetric Substrates – BCIP/NBT

  BioFX BCIP/NBT Membrane Substrate (Blue)
  BioFX BCIP/NBT Membrane Substrate (Purple)
TMB Microwell Kinetic Options

- Super Sensitive - TMBS
- Conductivity - TMBC
- Standard TMB - TMBW
- Slow Kinetic - TMSK
- Super Slow - TTMB
- Double Slow - TMDS
AP Colorimetric Substrate for Superior Performance

- SurModics’ BioFX® p-NPP with Stabilizing Pellets
  - Patented stabilization system
    - Stable for 2 years at 2°C - 8°C
  - One component, ready to use formulation
**p-NPP Microwell Substrate Kinetics**

Increased Substrate Kinetics over Competitors
$p$-NPP Competitor Study

Improved Signal to Noise over competitors at 28 days.
Numerous Secondary Antibodies

Numerous host and cross-absorption options
- Mouse
- Human
- Swine
- Goat
- Rat
- Hamster
- Chicken
- Sheep

Available in multiple formats
- Purified
- Horseradish peroxidase (HRP)
- Alkaline phosphatase (AP)
- Rhodamine (TRITC)
- Fluorescein (FITC)
- Biotin (BIOT)
- Texas Red (TXRD)
Microarray Slides: CodeLink® with TRIDIA™ Surfaces

• Types
  – CodeLink Activated Slides
  – CodeLink HD Activated Slides
  – TRIDIA EP (Epoxy) Slides

• Bind DNA, RNA, proteins, antibodies, peptides, microRNA, siRNA

• Benefits
  – In-solution like hybridization for optimal sample binding
  – Thin hydrophilic polymer coating for prevention of nonspecific binding and low background
  – NHS ester reactive or epoxide reactive groups
TRIDIA™ Surface Chemistries

– Hetero-trifunctional polymers
  • a photoactive group
  • passivating polymer backbone
  • customizable reactive groups

Reacting group

Passivating polymer backbone

Photoactive group
Microarray Slides: New TRIDIA™ Epoxy Surface

Experiment 1: Blocking with BSA

<table>
<thead>
<tr>
<th>ng/ml troponin</th>
<th>TRIDIA EP</th>
<th>Competitor E</th>
<th>Competitor T</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>![Image]</td>
<td></td>
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<tr>
<td>1</td>
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<td>3</td>
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<tr>
<td>10</td>
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<tr>
<td>30</td>
<td>![Image]</td>
<td></td>
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<tr>
<td>80</td>
<td>![Image]</td>
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</tr>
<tr>
<td>120</td>
<td>![Image]</td>
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<td></td>
</tr>
</tbody>
</table>

No primary Ab added

Experiment 2: Blocking without BSA

<table>
<thead>
<tr>
<th>TRIDIA EP</th>
<th>Competitor E</th>
<th>Competitor T</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image]</td>
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</tr>
</tbody>
</table>

- Passivating surface
- Effective blocking
- Higher S/N ratios
• Recombinant Autoantigens

  – Human recombinant autoantigens represent DIARECT's major product line. They are used for the development and production of autoimmunity diagnostic kits. Whether in ELISAs, dot or blot methods, our antigens have a proven track record for quality and sensitivity.

  – Clinical Associations:
    > Thyroid Autoimmune Disease
    > Nuclear Antigens (ANA / ENA)
    > ANCA Associated Disease
    > Anti-Phospholipid / Thromboembolic Syndrome
    > Autoimmune Hepatitis and Liver Disease
    > Goodpasture Syndrome
    > Gastrointestinal / Celiac Disease
    > Human Anti-dsDNA mAb Standards
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- DIARECT autoimmune antigens
- BioFX secondary antibodies
- BioFX blocks, diluents, wash solutions, and stop reagents
SurModics Technical Support Team

- Scientists available with technical expertise across all IVD areas.
- IVD group solves problems.
- Technical questions = immediate response.
- Willingness to break down your assay.
- If you can’t find a product, let us know, your question could be our next new product.
- We would be happy to meet you and discuss any and all assay questions.