Tools for Enhancement of Diagnostic Immunoassay Applications

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Abstract
Optimization of immunoassay applications is often troubled by issues such as non-specific binding, matrix interferences, destabilization of antibody-antigen interactions, and limited sensitivity. SurModics offers tools to address these problems and provides the framework to develop sensitive, reproducible, and robust immunoassays. Novel stabilizer formulations have been developed to provide protein stability in both the dried state as well as in solution. The data presented here show improved stability compared to both common in-house formulations as well as other competitor solutions.

Protein Stability

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>% Retained Efficacy Day 0</th>
<th>% Retained Efficacy 7 days</th>
<th>% Retained Efficacy 1 month</th>
<th>% Retained Efficacy 6 months</th>
<th>% Retained Efficacy 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>StabilCoat</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>StabilGuard</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>1X PBS + 1%BSA</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>1X PBS + 1%BSA + 0.01% Trypsin</td>
<td>60</td>
<td>60</td>
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</tbody>
</table>

Figure #1: Dried antibody stability with StabilCoat and StabilGuard stabilizers

Methods: A capture antibody was stabilized with different commercially available stabilizers. This accelerated stability study challenged the captured antibody at 37°C. The retained activity of the captured antibody was evaluated in a sandwich ELISA over one year by comparing the immunoassay signal produced by the 4°C control versus 37°C. Retained activity values are determined by dividing the average optical density at 37°C by the average optical density at 4°C, then multiplying by 100.

Results: 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.

Microarray Applications

Figure #4: Attachment of HUVEC in a Cell Array

Methods: RGD peptide was printed on CodeLink® slides (SurModics) and incubated overnight at 75% humidity. Slides were washed and stained by exposure to UV light. Slides were then placed in sterile 100 mm dishes (1 slide/dish), and human umbilical vein endothelial cells (HUVEC; Lonza) were seeded at 30,000 cells/mL containing growth media (Lonza) at 20°C. After 3 days of proliferation, HUVECs were stained with 1 µg/mL calcein AM (Molecular Probes). Slides were then fixed, mounted, and imaged using FITC and DAPI filter sets.

Results: The CodeLink surface bound RGD peptide promoted cell adhesion and eliminated non-specific binding of cells to the remainder of the surface. Image A is a fluorescent image of 6 RGD spots on a CodeLink slide. Image B is a magnified spot on the array highlighting the specific attachment of the cells to the spot where RGD was printed. Image C is a phase contrast image of the same spot.

Summary

- Superior protein stability/activity in both dried and in-solution formats
- Enhanced signal and eliminated cross-reactivity concerns on membranes
- Improved signal-to-noise ratios and decreased non-specific binding on nitrocellulose arrays
- TRIDIA surface chemistry provides:
  - Support for cell array formats
  - Superior epoxy surface
  - Simultaneous biomolecule immobilization and passivation

Demonstrated Improved Assay Performance Across Multiple Diagnostic Applications!

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