

# Binding Capacity of Streptavidin Microspheres

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## ProActive® Streptavidin Coated Microspheres and Their Binding Capacity for Biotin and Biotinylated Oligonucleotides

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\* Presented as part of the OEM Lecture Series, AACC, San Francisco, CA, 2000.

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- Introduction
- Product Development
- Product Characterization
- Product Comparison
- Conclusion

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### Introduction Binding Conjugates

ProActive® Microspheres

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### Introduction Streptavidin-Biotin Interaction

- High binding affinity ( $K = 10^{13}$ )
- Stable over wide ranges of temperature and pH
- Commercial availability of biotinylated ligands

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### Introduction Applications

**Immunology (solid phase immunoassays)**

- IGEN System
- Beckman Access
- Nichols Advantage
- ...

**Molecular Biology (oligo dT)**

- Nucleic acid synthesis
- Purification
- Hybridization
- ...

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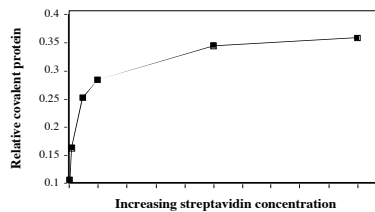
### Development Goals

- Stability
- Reactivity
- Specificity
- Reproducibility

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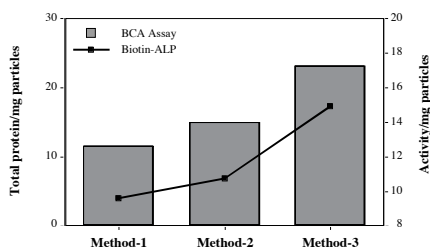
### Development Process Optimization



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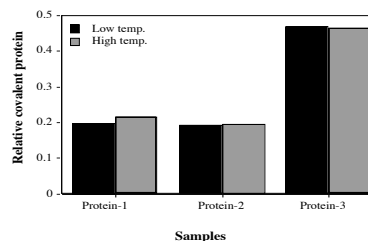
### Development Coating Process Choice



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### Development Process Optimization



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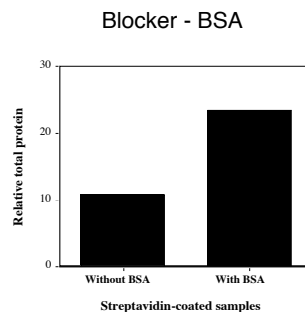
### Development Process Optimization

- Carrier molecule
- Linker
- pH
- Particle surface
- EDAC
- Streptavidin
- Temperature
- Blocker
- ...

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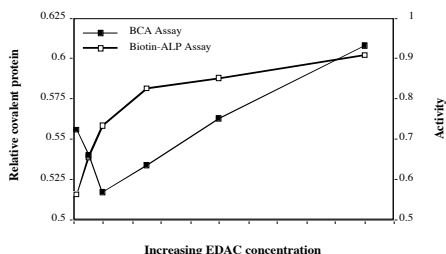
### Development Process Optimization



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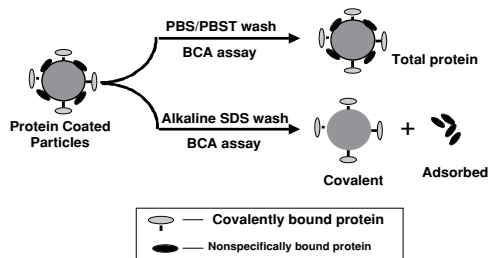
### Development Process Optimization



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### Characterization Bound Protein Determination

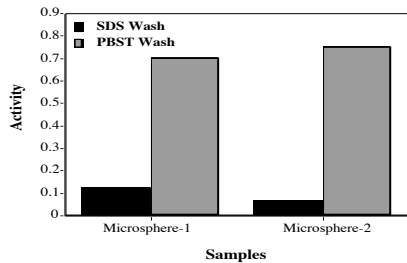


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### Characterization Bound Protein Determination

#### Binding Activity

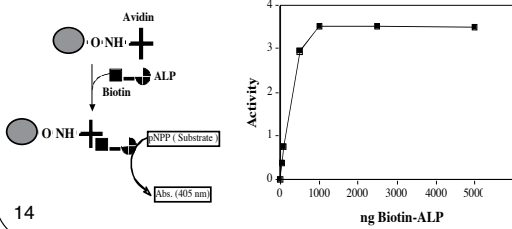


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### Characterization Activity

#### Non-competitive Enzymatic Assay Biotin-Alkaline Phosphatase (B-ALP)

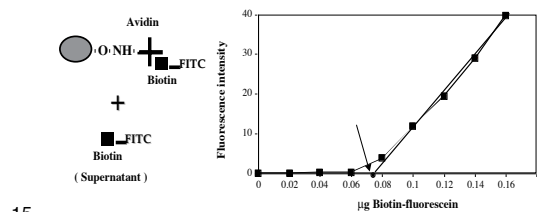


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### Characterization Activity

#### Non-competitive Fluorescence Assay Biotin-Fluorescein (B-FITC)

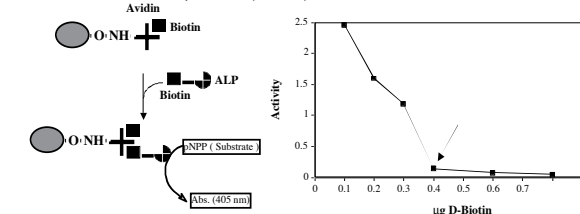


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### Characterization Activity

#### Non-competitive Enzymatic Assay Biotin-Alkaline Phosphatase (B-ALP)

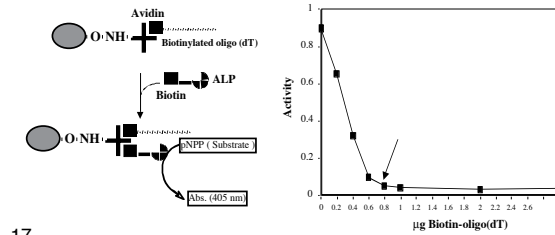


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### Characterization Activity

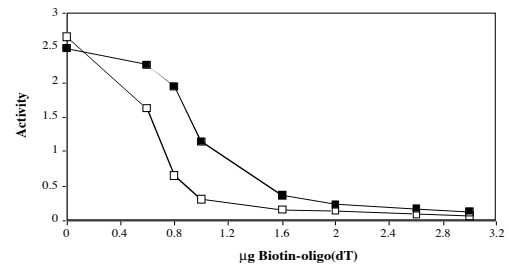
#### Competitive Enzymatic Assay B-ALP and Biotinylated oligo dT



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### Comparison Streptavidin Source

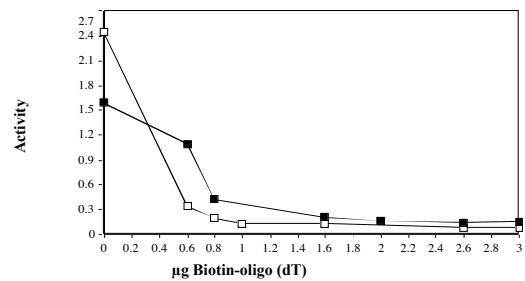


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Competitive assay: biotinylated oligo (dT) and biotin-ALP

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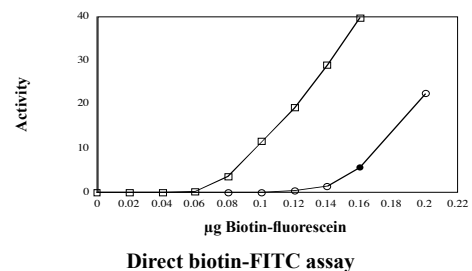
### Comparison Microsphere Type



Competitive Assay: biotinylated oligo (dT) and biotin ALP

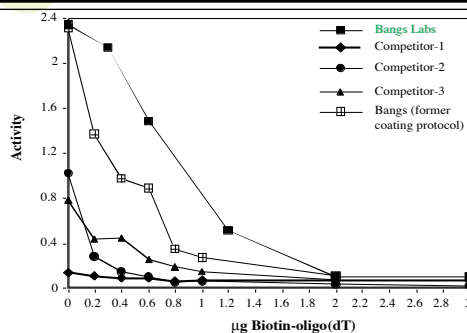
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### Comparison Microsphere Size



Direct biotin-FITC assay

## Comparison Vendors



21  Competitive assay: biotinylated oligo (dT) and biotin-ALP  
 Normalized by solids content

## Conclusion

ProActive®

Process Optimized

- EDAC concentration
- Streptavidin concentration
- Incubation temperature
- BSA blocking

Assays

- Protein
- Activity

Outsourcing

- Normalize solids content
- Binding capacity
- Binding capacity assay

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