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HUMAN HEALTH | ENVIRONMENTAL HEALTH

Creating an AlphaLISA® Assay With Your Own Antibodies

An Overview and Key Points to Consider

Creating an AlphaLISA Assay – Table of Contents

Overview of assay principles and configurations

- Working range of binding affinities
- The four basic assay configurations
- Available choices of donor and acceptor beads

Key considerations in setting up your assay

- Antibody selection
- Order of antibody addition

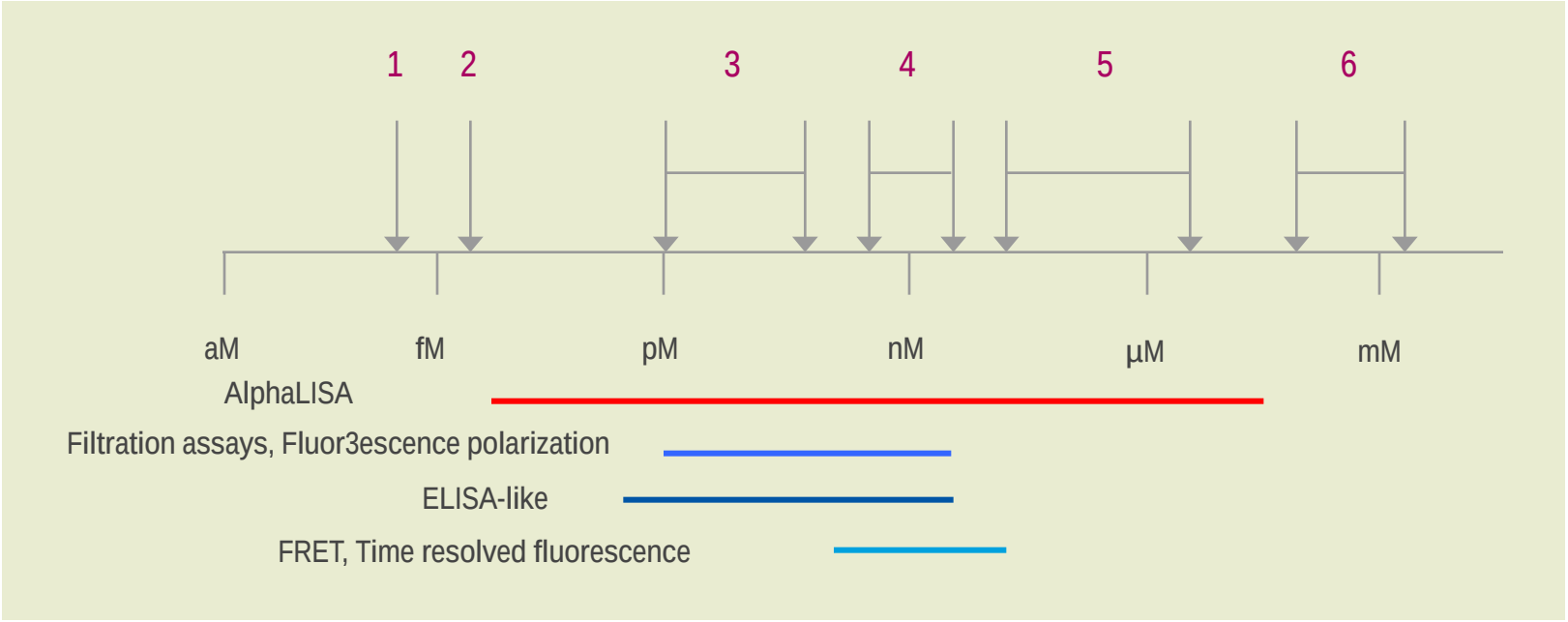
Additional resources

- AlphaLISA Assay Development Guide
- Technical support
- OnPoint custom reagent and assay development services

Creating an AlphaLISA Assay - Overview

AlphaLISA assays have a broad working range in terms of binding affinity

1) highest biological interaction
2) biotin-streptavidin
3) very high affinity antibody, receptor ligands
4) most antibodies of good quality
5) most protein-protein interactions
6) lectins



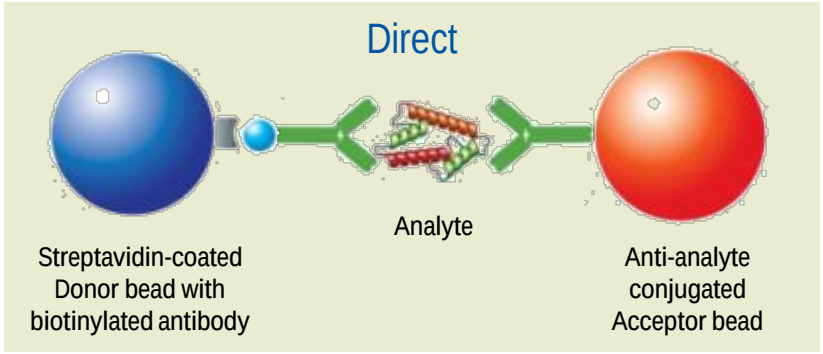
AlphaLISA is more flexible than other assay platforms

Creating an AlphaLISA Assay - Overview

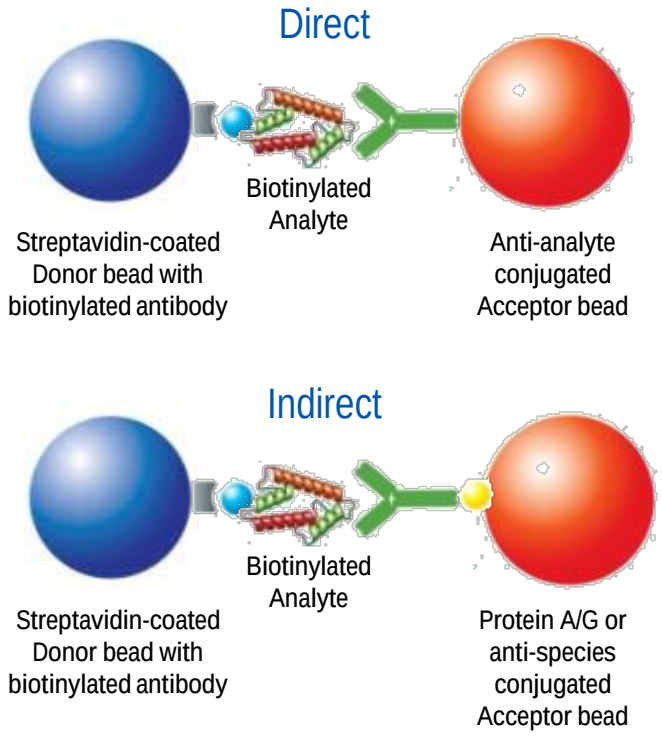
Understand the four basic configurations of AlphaLISA assays.

- Standard versus Competition assay configuration
- Direct versus Indirect coupling to acceptor beads

Standard assay



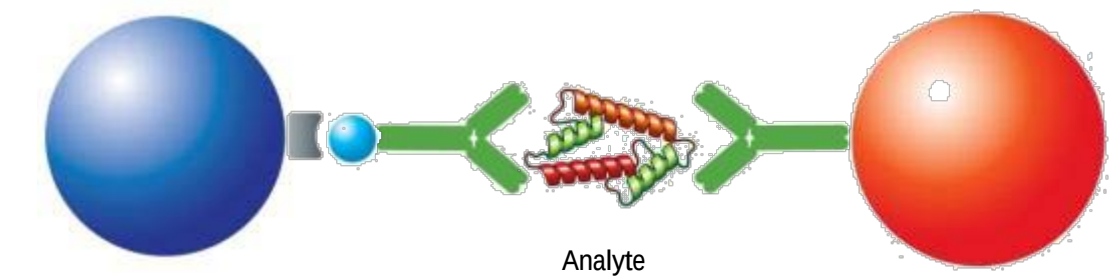
Competition assay



Standard versus Competition AlphaLISA assay configurations

Standard

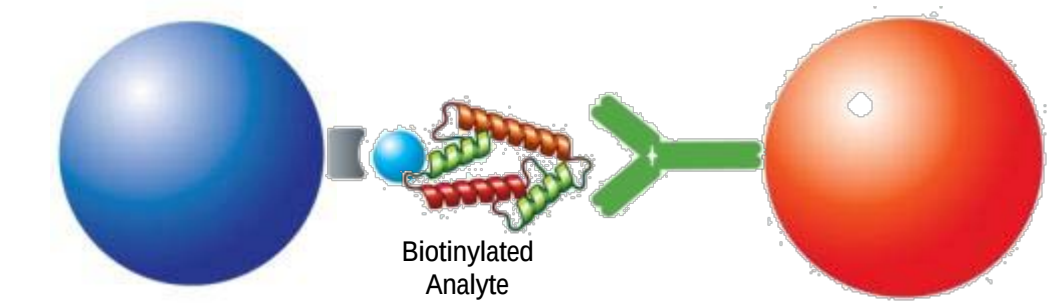
- The standard configuration is a sandwich assay employing two different antibodies that recognize non-overlapping epitopes on the target molecule
- Increasing amounts of target in the sample result in higher luminescent signals
- The target molecule is not coupled to biotin



Standard versus Competition AlphaLISA assay configurations

Competition

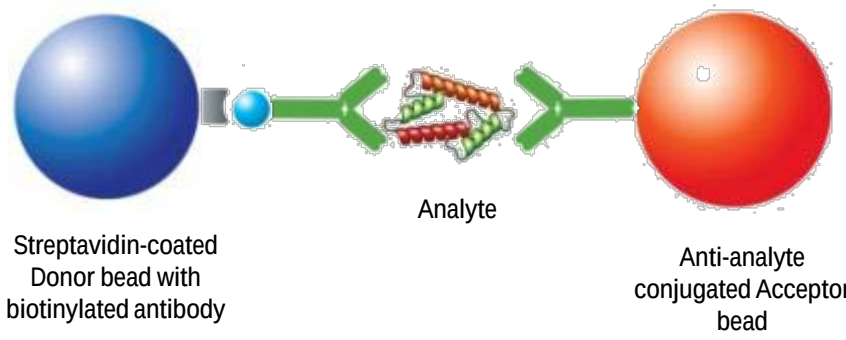
- The competition assay configuration is used when only one specific antibody is available to the target molecule
- A purified source of the target molecule is labeled with biotin and added to the assay in a known concentration
- Increasing amounts of unlabeled target in the sample compete with binding of the biotin-labeled target and result in a decrease in signal

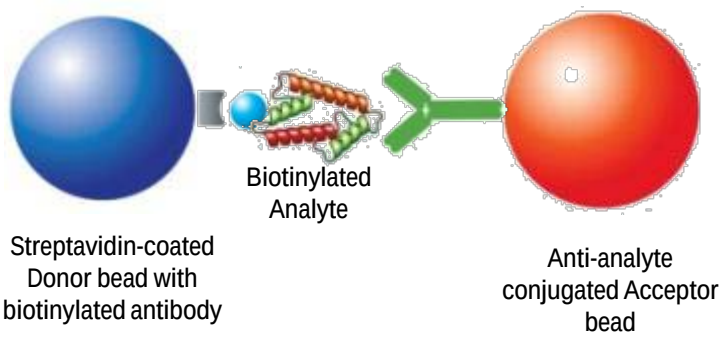


Direct versus Indirect AlphaLISA assay configurations

Direct

- The acceptor beads are directly coupled to the target-specific antibody
- These assay configurations are simpler to optimize and perform than the indirect configurations

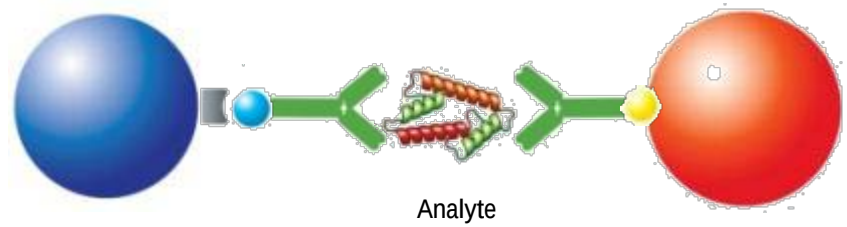


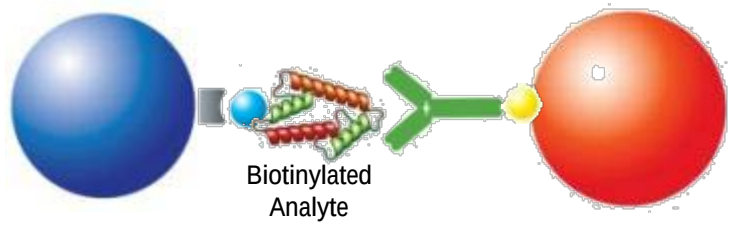


Direct versus Indirect AlphaLISA assay configurations

Indirect

- The acceptor beads are not directly coupled to the target-specific antibody. Instead, they are coupled to protein A or another antibody-binding moiety
- The target-specific antibody is captured on acceptor beads during an incubation step
- This assay configuration is sometimes used when the target-specific antibody is available only in limited amounts (too small to allow a coupling reaction to beads)
- These assay configurations are somewhat more complicated to optimize and perform than the direct configurations





Creating an AlphaLISA Assay - Key Considerations

A wide range of donor and acceptor bead choices are available for AlphaLISA assays

- Streptavidin donor and acceptor beads
- Glutathione donor and acceptor beads
- Nickel chelate donor and acceptor beads

- Anti-goat IgG (Fc specific) acceptor beads
- Anti-human IgG (Fc specific) acceptor beads
- Anti-mouse IgG (Fc specific) acceptor beads
- Anti-rabbit IgG (Fc specific) acceptor beads
- Anti-rat IgG (Fc specific) acceptor beads
- Protein A acceptor beads
- Protein G acceptor beads

- Unconjugated acceptor beads
- Anti-DIG acceptor beads
- Anti-FLAG acceptor beads
- Anti-GST acceptor beads
- Anti-c-myc acceptor beads

Creating an AlphaLISA Assay - Key Considerations

Antibody selection criteria

Antibodies must recognize non-overlapping epitopes on the target molecule

Obtain and test as many antibodies as possible

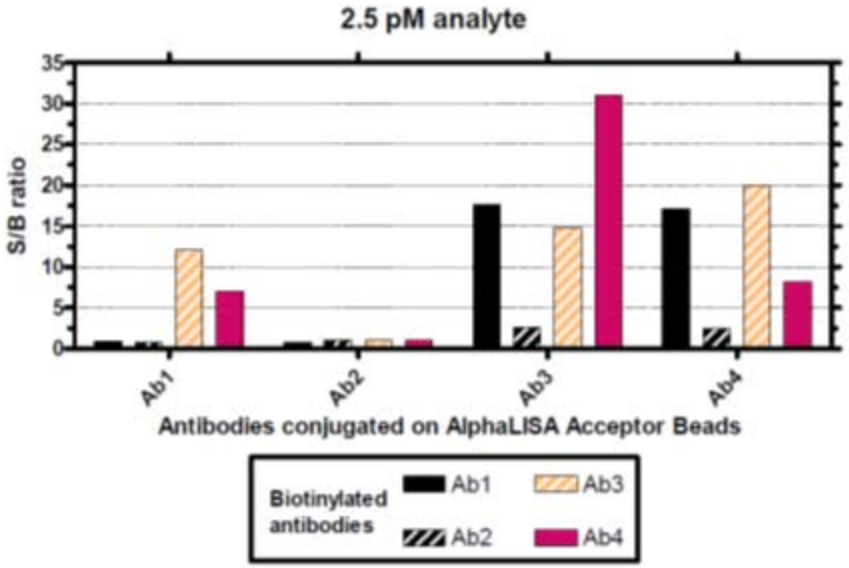
When using protein A-conjugated acceptor beads (as in an indirect assay), only one of the two antibodies in the assay is of a type bound by protein A

Protein A binds with varying affinity to different classes of antibody

- High affinity binding - to human IgG1, IgG2 and to mouse IgG2a, IgG2b
- Moderate affinity binding - to human IgM, IgA, IgE and to mouse IgG3, IgG1
- No binding - to Fab fragments and to mouse IgM, IgA, IgE+

Antibody selection and order of antibody addition

- For each antibody, prepare two conjugates – one to biotin and one to acceptor beads
- Test all pair-wise combinations of biotinylated and bead-conjugated antibodies
- Selection of the best antibody combination and order of addition can greatly affect the assay sensitivity



In this example, Ab3-conjugated acceptor beads and biotinyated-Ab4 gave the highest S/B (signal:background) ratio.

AlphaLISA Technical Support

AlphaLISA Assay Development Guide

- Explanation of assay principles
- Detailed workflow and protocols for optimizing assays
- Explanation of data analysis
- Visit http://las.perkinelmer.com/TechnicalSupport/default.htm to download pdf file.

Technical Support

North America

Phone: (800) 762-4000
After the prompt: 3, then 2

Techsupport@perkinelmer.com

Europe

Techsupport.europe@perkinelmer.com

OnPoint custom reagent and assay development services

- Custom conjugation of ligands, proteins and antibodies to biotin, beads, or other haptens
- Complete assay development and validation services