AlphaLISA Immunoassay Conversion Quick Start Guide

AlphaLISA immunoassays offer advantages over other immunoassay formats as AlphaLISA assays do not require any wash steps, have fewer protocol steps, require less sample, and provide a large dynamic range. This quick guide presents a fast proof-of-concept experiment to easily convert an immunoassay to an AlphaLISA assay. The quickest way to convert an existing immunoassay is to use an indirect AlphaLISA immunoassay format. This format uses pre-coated Alpha Donor beads and AlphaLISA Acceptor toolbox beads to bind your two sandwiching antibodies.

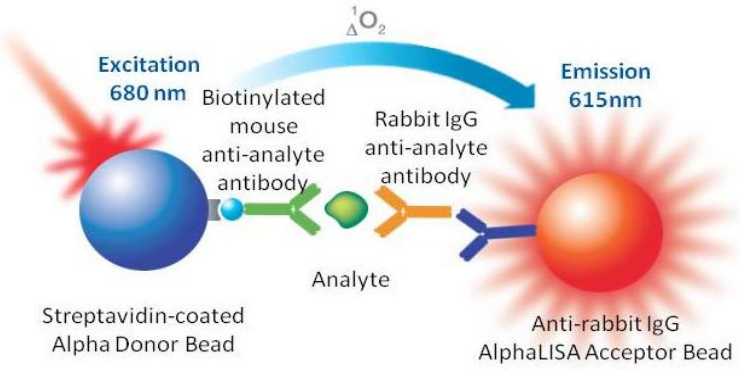

Figure 1. Indirect AlphaLISA immunoassay format. One of the sandwiching antibodies (in this case, a biotinylated mouse antibody) is captured by streptavidin Alpha Donor beads. The second sandwiching antibody (in this case, a rabbit IgG antibody) is captured by anti-rabbit IgG AlphaLISA Acceptor beads. When analyte is present in your sample, the Donor and Acceptor beads come into proximity. Excitation of the Donor beads results in emission of light from the AlphaLISA Acceptor beads. The signal generated is proportional to the amount of analyte present in the sample.

A variety of pre-coated bead products are available for your assay. Refer to our complete list of available beads.

Table 1. Alpha Donor and AlphaLISA Acceptor beads for immunoassay design.

Bead coating	Toolbox Alpha Donor beads	Toolbox AlphaLISA Acceptor beads
Streptavidin	6760002	AL125
***Strep*-Tactin®**	AS106	AL136
Protein A	AS102	AL101
Protein G		AL102
Protein L		AL126
Anti-rabbit IgG	AS105*	AL104*
Anti-mouse IgG	AS104*	AL105*
Anti-human IgG		AL103*
Anti-rat IgG		AL106*
Anti-goat IgG		AL107*
Anti-sheep IgG		AL132*
Anti-mouse IgM		AL130
Anti-chicken IgY		AL131

Unconjugated	6762013	6772001

* Fc-specific antibody

There are two main considerations in selecting appropriate Donor and Acceptor beads for your assay:

1. You need to make sure that each antibody can only associate with either the Alpha Donor bead, or the AlphaLISA Acceptor bead. If one of your antibodies can bridge both the Donor and Acceptor bead in your assay, you may see high background.
2. Additionally, you need to make sure that your Donor and Acceptor beads cannot bind each other (in the absence of analyte and sandwiching antibodies). For more information on bead selection, including bead pairings that can cross-react, please refer to www.perkinelmer.com/beadselection.

Before you begin

- The Donor beads used in Alpha assays are somewhat light sensitive. We recommend working under subdued lighting conditions when working with the beads (less than 100 Lux - the level of light produced on an overcast day). For example, you can turn half of the laboratory lights off and work at a bench away from windows and where the overhead light is not on. Incubate the plate in the dark (for example, placing the covered or sealed plate in a drawer).
- The Alpha signal is temperature-dependent. If you will be performing incubations at 37°C or other temperatures, we recommend that you equilibrate the plate back to room temperature before reading to ensure signal uniformity across the plate.
- Alpha assays require a special reader capable of measuring an Alpha assay, such as an EnVision® or EnSpire™ Multi-label Plate Reader. Many standard time-resolved fluorimeters and luminometers cannot read Alpha assays.
- We recommend preparing only what you need for the day's experiments. Do not store working dilutions of beads for more than one day.
- We recommend using white plates (such as the PerkinElmer 96-well ½ AreaPlate™, Cat. No. 6005560), or light grey AlphaPlates™ (Cat. No. 6005390 or 6004350), for these assays.

Step One: Antibody cross-titration

A common first experiment is to titrate each binding antibody in a cross titration matrix on the plate. Titration of one partner down the plate and the other partner across the plate allows a view of all the combinations and includes the important controls of no antibodies and single antibodies only. For this assay, we will titrate each antibody from 0 – 3 nM. Typically, the optimal antibody concentrations will fall in the 1-3 nM range (although there are some examples with higher or lower concentrations, depending on affinity to the analyte).

1. Reagent preparation

1.1 Prepare 1X AlphaLISA Immunoassay Buffer (Cat. No. AL000)

Dilute 0.6 mL of 10X AlphaLISA Immunoassay Buffer in 5.4 mL of deionized H_2O

1.2 Preparation of sandwiching Antibody #1

a. Prepare a 500 nM working solution of Antibody #1.
b. Prepare 5X dilution series:

Tube	Vol of antibody	1X AlphaLISA Immunoassay Buffer	[Intermediate] (nM)	[Final Assay] (nM)
1	15 µL of 500 nM Antibody #1	485 µL	15	3
2	100 µL of tube 1	200 µL	5	1
3	50 µL of tube 1	450 µL	1.5	0.3
4	300 µL of buffer		0	0

1.3 Preparation of sandwiching Antibody #2

a. Prepare a 500 nM working solution of Antibody #2

b. Prepare 5X dilution series:

Tube	Vol of antibody	1X AlphaLISA Immunoassay Buffer	[Intermediate] (nM)	[Final Assay] (nM)
A	15 µL of 500 nM Antibody #2	485 µL	15	3
B	100 µL of tube A	200 µL	5	1
C	50 µL of tube A	450 µL	1.5	0.3
D	300 µL of buffer		0	0

1.4 Preparation of standard analyte:

a. Prepare 10X working solution (30 ng/mL) of analyte in 1X AlphaLISA Immunoassay buffer. (The final concentration of analyte in your assay will be 3 ng/mL.)

2. Prepare 4X working solution (40 µg/mL) of AlphaLISA Acceptor beads:

Lightly vortex AlphaLISA Acceptor beads

Add 10 µL Acceptor beads (5 mg/mL) to 1240 µL 1X AlphaLISA Immunoassay buffer and mix thoroughly

3. (During 2nd incubation – refer to protocol below): Prepare 4X working solution (160 µg/mL) of Alpha Donor beads:

Lightly vortex Alpha Donor beads

Add 32 µL Donor beads (5 mg/mL) to 968 µL buffer 1X AlphaLISA Immunoassay buffer and mix thoroughly

Protocol for 50 µL assay in white 96-well ½ AreaPlate™
(*all reagents diluted in 1X AlphaLISA Immunoassay Buffer, Cat. No. AL000)

Add 10 µL Antibody #1 (0, 0.3 nM, 1 nM, and 3 nM final concentrations in 50 µL reaction)

⇩

Add 10 µL Antibody #2 (0, 0.3 nM, 1 nM, and 3 nM final concentrations in 50 µL reaction)

⇩

Add 5 µL analyte (5 ng/mL final concentration in 50 µL reaction, or 1X AlphaLISA ImmunoAssay Buffer alone – refer to plate map)

⇩

Incubate at 23°C for 60 minutes

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Add 12.5 µL of AlphaLISA Acceptor beads (10 µg/mL final concentration in 50 µL reaction)

⇩

Incubate at 23°C for 60 minutes

⇩

Add 12.5 µL Alpha Donor beads (final concentration 40 µg/mL in 50 µL reaction)

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Incubate in dark at 23°C for 60 minutes

⇩

Read on an Alpha-capable instrument (EnVision® or EnSpire® Multilabel plate readers)

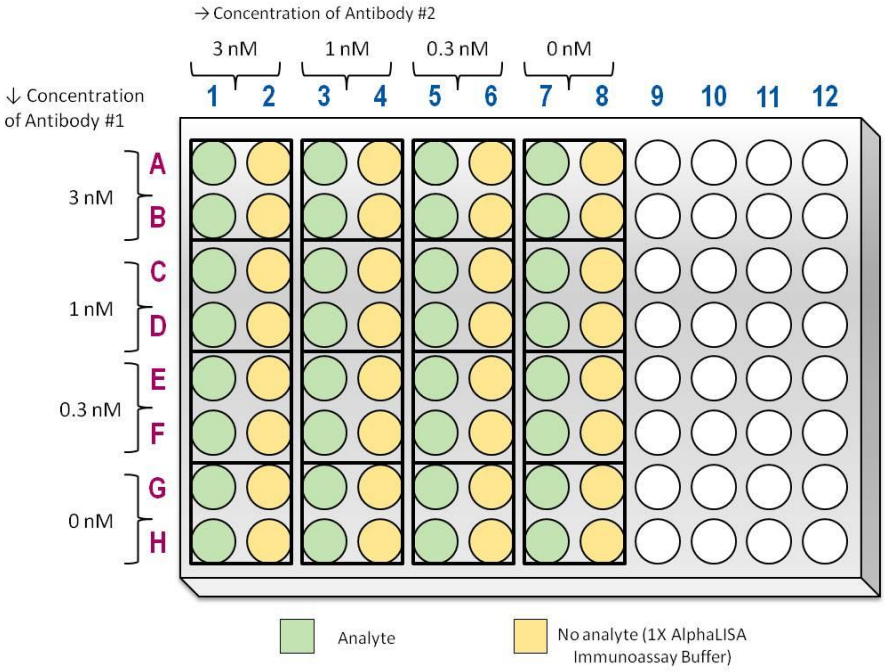

Figure 2. Plate map for antibody cross-titration assay in white 96-well ½ AreaPlate (Cat. No. 6005560). The concentration of Antibody #1 will be titrated down each row, and the concentration of Antibody #2 will be titrated across the columns. This will create a matrix of 16 different combinations of antibody concentrations (refer to black, square outlines).

Data evaluation and analysis

For the plate map shown in Figure 2, data is obtained in duplicate. You will first determine signal-to-background (S:B) for each of the 16 antibody concentration combinations by dividing the "analyte" sample (green wells) by the "no analyte" sample (yellow wells). Then plot results as a 3-D bar chart, as shown in Fig. 3. When evaluating your results you should consider the signal-to-background as well as the cost of your antibodies. For these results, a reasonable choice would be 1 nM Antibody #1 paired with 0.3 nM Antibody #2. A typical second experiment may be to run a full titration of analyte using these parameters to determine the dynamic range of your AlphaLISA assay.

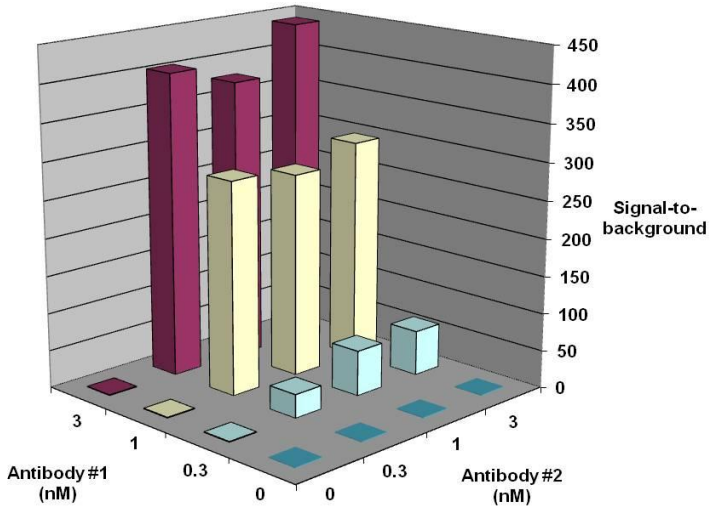

Fig 3. Results from antibody cross-titration experiment. Data is plotted as a 3-D bar chart using Microsoft® Excel®.

Further optimization steps

This quick start guide provides a fast proof-of-concept assay for AlphaLISA conversion. You have the option to further optimize your assay by changing parameters described in the table below. For more information on how to further optimize your AlphaLISA immunoassay, refer to the complete "AlphaLISA immunoassay conversion guide".

AlphaLISA Optimization	Comments
Change configuration from an indirect assay format to a direct assay format (recommended)	The most common configuration for an AlphaLISA sandwiching immunoassay is to use a biotinylated antibody in combination with streptavidin Donor beads, with your second antibody directly-conjugated to the AlphaLISA Acceptor bead. This assay configuration is least prone to interference from your sample or treatments.
Order-of-addition (recommended)	The protocol presented above is a 3-step protocol (incubation of sample/analyte with your two sandwiching antibodies, followed by addition of the AlphaLISA Acceptor beads, followed by addition of the Alpha Donor beads). Other order-of-addition protocols are also possible, and can influence the sensitivity and dynamic range of the assay.
Bead titration (optional)	Suggested range: 10 µg/mL to 40 µg/mL of Donor or Acceptor bead Start with: A cross-titration matrix, varying the concentration of Donor bead across each column (10, 20, 30, and 40 µg/mL) and Acceptor bead down each row (10, 20, 30, and 40 µg/mL). This will create 16 combinations of Donor and Acceptor beads.
Incubation time (optional)	Suggested range: 30 minutes – 2 hours per incubation step Start with: 60 minute incubation steps Incubation times may need to be lengthened (or can sometimes be shortened) depending on the kinetics of binding of your antibody to the analyte. Quick interactions (for example, binding of streptavidin to biotin) may only require a 15 or 30 minute incubation to reach equilibrium. Slower interactions may require longer times for association.
Buffer for diluting beads and biotinylated antibody (optional)	Start with: 1X AlphaLISA Immunoassay Buffer (Cat. No. AL000) For most assays, 1X AlphaLISA Immunoassay Buffer will result in best performance. This buffer contains casein, dextran, and detergent. If your background is high following assay

	optimization, you may try 1X HiBlock Buffer (Cat. No. AL004) instead. This buffer contains BSA and gelatin as additional buffer components.
Assay volume (optional)	Start with: 50 µL final volume in white 96-well ½ AreaPlate (Cat. No. 6005560) The assay can easily be miniaturized to 384-well or 1536-well format by simply proportionally decreasing the volumes of each addition step. The concentrations of each reagent should remain the same.