Development of *Strep*-Tactin®-conjugated Alpha Donor and AlphaLISA Acceptor beads as new tools for homogenous custom-based protein-protein interaction assays

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1 Abstract

Alpha and AlphaLISA® Toolbox products are stand-alone bead reagents developed to enable custom building of assays. These reagents have been used to study a wide variety of biochemical and cellular events including protein-protein interactions.
This work presents the development of *Strep*-Tactin®-conjugated Alpha Donor and AlphaLISA Acceptor beads. The *Strep*-Tactin®/*Strep*-tag® system is a unique protein purification and detection system based on high selectivity of the engineered streptavidin mutant *Strep*-Tactin® for *Strep*-tag® peptide tags, which can be introduced within the sequence of any recombinant protein. *Strep*-Tactin® was conjugated to Alpha Donor beads or AlphaLISA Acceptor beads and tested in an AlphaLISA assay using double tagged protein probe (*Strep*-tag II-Azurin-6xHis and One-STrEP tag-Azurin-6xHis). Following optimization of the assay parameters (e.g. order of addition of the assay reagents), results show that *Strep*-Tactin®-conjugated beads recognize *Strep* tag II-tagged proteins with nearly 100-fold higher apparent affinity compared to streptavidin-conjugated equivalents, while the apparent affinity for biotin-labeled proteins is the same for both streptavidin and *Strep*-Tactin®-conjugated beads. In addition, Alpha Donor beads and AlphaLISA Acceptor beads conjugated to *Strep*-Tactin® can be used in the presence of cell lysates or some cell culture media without impacting assay performance parameters, such as EC50 and signal-to-noise ratio. Overall, *Strep*-Tactin®-conjugated beads, together with other new Toolbox products (pre-coated beads able to capture common protein affinity tags such as FLAG®, MBP, 6xHis, or FITC, as well as beads conjugated with several anti-species antibodies) are versatile tools for custom-based homogenous interaction assays.

2 Introduction

In an AlphaLISA protein-protein interaction assay, one protein is captured on the Alpha Donor beads, while the other protein is captured on the AlphaLISA Acceptor beads. When the two proteins interact, the Donor bead is brought into proximity of the Acceptor bead. Excitation of the Donor bead at 680 nm provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor bead, resulting in a sharp peak of light emission at 615 nm.

Depending on the assay, beads can be selected from a panel of Toolbox products, which includes the new *Strep*-Tactin®-coated Alpha Donor beads and *Strep*-Tactin®-coated AlphaLISA Acceptor beads. *Strep*-Tactin®, an engineered Streptavidin mutant, binds with high selectivity *Strep*-Tag® II, a 8 amino-acid peptide tag, and One-STrEP-tag, which contains two *Strep*-Tag® II peptide sequences separated by a linker. The *Strep*-Tactin®/*Strep*-tag® system has been widely used in protein purification and more recently in protein detection.

For any Toolbox product, the quality of the bead throughout Development as well as during Production is being monitored by a functional evaluation assay. A typical evaluation assay involves a probe which is captured by both the Acceptor and Donor beads simultaneously, leading to the generation of an AlphaLISA signal .

3 Material

AlphaLISA Acceptor beads and Alpha Donor beads are available as stand-alone Toolbox products from PerkinElmer. *Strep*-Tactin® is manufactured by IBA GmbH. and the related patents are held by IBA. PerkinElmer has the non-exclusive right to coat AlphaLISA and Alpha beads with *Strep*-Tactin®.

AlphaLISA Acceptor beads: Streptavidin (AL125), anti-6xHis (AL128), anti-GFP (AL133), *Strep*-Tactin® (AL136)
Alpha Donor beads: *Strep*-Tactin® (AS106), Streptavidin (6760002), anti-mouse IgG (AS104)
AlphaLISA Universal Assay Buffer: AL000; AlphaLISA Lysis buffer: AL003
Other reagents: Biotin-rabbit IgG (Jackson ImmunoResearch, 011-060-003); GFP (abcam, ab84191; in-house biotinylation), azurin (all forms, IBA, custom order), RIPA lysis buffer (Pierce, 89900)

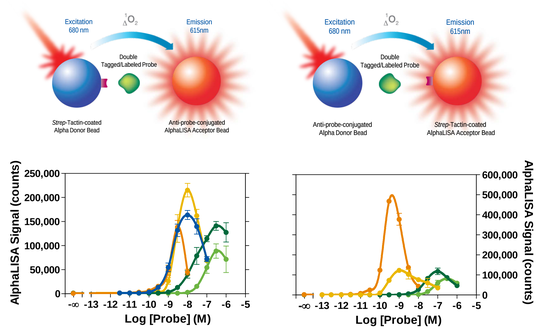
4 Methods

Summary of different experimental set-ups performed; all experiments were carried in a white OptiPlate™-384 microplate:

Typical (Buffer & Cell culture Medium*)	Cell Lysate#	Antibody Screening
	5 µL of cell lysate	
15 µL of 1.7X probe dilution	10 µL of 2.5X probe dilution	10 µL of 2.5X probe dilution
5 µL of 5X AlphaLISA Acceptor beads§ (20 µg/mL final)		
		Incubate 30 min at 23ºC
		5 µL of 5X mouse anti-His Ab
Incubate 60 min at 23ºC		
5 µL of 5X Alpha Donor beads (20 µg/mL final)		
Incubate 30 min at 23ºC in the dark		Incubate 60 min at 23ºC in the dark
Read using EnSpire® or EnVision® Multilabel Alpha Reader		

* All cell culture media were diluted six-times with the Universal buffer prior to performing serial dilution of a probe in order to obtain 10% final concentration of cell culture media/well.
# $4x10^6$ cells were lysed in 1 mL of lysis buffer
§ *Strep*-Tactin®-coated AlphaLISA Acceptor beads were added to the reaction mixture prior the last incubation step unless specified otherwise.

5 Characterization of *Strep*-Tactin®-Coated Beads

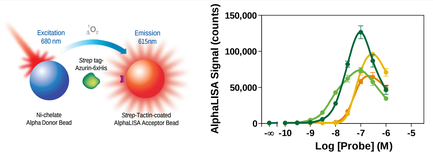


Probe	Acceptor bead	EC_{50} (nM)	S/B
Strep-Tag II-Azurin-6xHis	Ni-chelate	73.9	80
One-STrEP-Azurin-6xHis	Ni-chelate	22.5	114
One-STrEP-Azurin-6xHis	Anti-6xHis	1.4	109
Biotin-rabbit-IgG	Protein A	2.3	136
Biotin-rabbit-IgG	Anti-rabbit IgG	1.2	180

Probe	Donor bead	EC_{50} (nM)	S/B
Strep-Tag II-Azurin-6xHis	Ni-chelate	59.6	90
One-STrEP-Azurin-6xHis	Ni-chelate	7.4	110
Biotin-rabbit-IgG	Protein A	0.2	134
Biotin-rabbit-IgG	Anti-rabbit IgG	0.1	246

Different set-ups of *Strep*-Tactin®-conjugated beads evaluation assays
Strep-Tactin®-coated Alpha Donor beads (left panel) and AlphaLISA Acceptor beads (right panel) were tested in an evaluation assay using various partner beads and three different probes (*Strep*-Tag® II-Azurin-6xHis, One-STrEP-Azurin-6xHis or Biotin-rabbit-IgG).

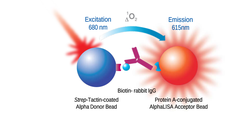
6 Order of Addition



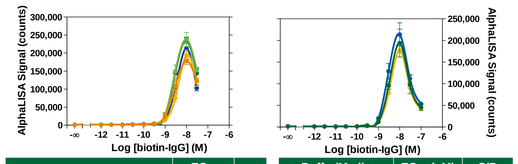
Probe	Order of addition	EC_{50} (nM)	S/B
Strep-Tag II-Azurin-6xHis	1-AB, 2-DB	46	89
One-STrEP-Azurin-6xHis	1-DB, 2-AB	61	110
Strep-Tag II-Azurin-6xHis	1-AB, 2-DB	7	109
One-STrEP-Azurin-6xHis	1-DB, 2-AB	18.9	139

Order of addition of *Strep*-Tactin®-coated AlphaLISA Acceptor beads
Strep-Tactin®-coated AlphaLISA Acceptor beads were added to the reaction mixture prior the first or second 23 C-incubation step. Different assay set-ups were tested using 3 probes (*Strep*-Tag II-Azurin-6xHis, One-STrEP-Azurin-6xHis or Biotin-rabbit-IgG (data not shown)).

7 Cell Lysates and Cell Culture Media



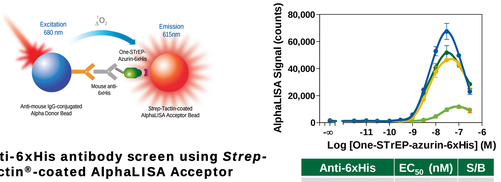
Effect of cell lysates and cell culture media on the performance of *Strep*-Tactin®-coated Alpha Donor beads.



Buffer/Lysates	EC_{50} (nM)	S/B
Universal buffer	2.5	133
AlphaLISA Cell Lysis buffer (AL)	2.4	197
RIPA Cell Lysis buffer (R)	3.5	194
HEK 293 in AL	2.3	222
HEK 293 in R	1.5	188

Buffer/Media	EC_{50} (nM)	S/B
Universal buffer	2.5	134
DMEM + 10% FBS	3.2	123
RPMI + 10% FBS	3.0	132

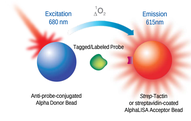
8 Application - Antibody Screening



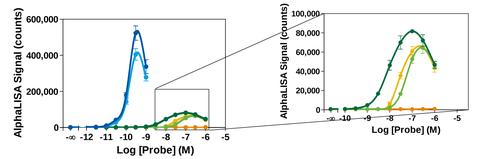
Anti-6xHis antibody screen using *Strep*-Tactin®-coated AlphaLISA Acceptor beads.
The ability of different anti-6xHis antibodies to recognized One-STrEP-Azurin-6xHis in an AlphaLISA assay was assessed using *Strep*-Tactin®-coated AlphaLISA Acceptor beads and Alpha anti-mouse IgG Donor beads.

Anti-6xHis	EC_{50} (nM)	S/B
Antibody 1	3.3	30
Antibody 2	8.3	9
Antibody 3	8.2	9
Antibody 4	3.8	35
Antibody 5	4.2	57

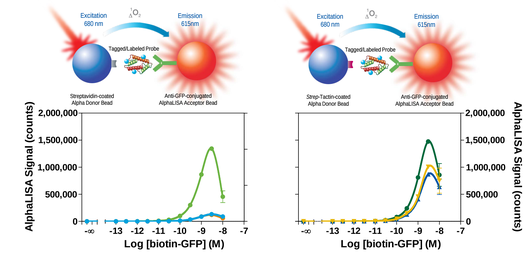
9 Comparison of *Strep*-Tactin® versus Streptavidin-Coated Beads



Performance of *Strep*-Tactin® vs. Streptavidin-coated Alpha Donor beads in evaluation assays.
Different assay set-ups were tested using 3 probes (*Strep*-Tag II-Azurin-6xHis, One-STrEP-Azurin-6xHis or Biotin-rabbit-IgG).



Acceptor bead	Streptavidin			*Strep*-Tactin®		
Donor bead	Ni-chelate	Ni-chelate	Anti-rabbit IgG	Ni-chelate	Ni-chelate	Anti-rabbit IgG
Probe	*Strep*-Tag II-Azurin-6xHis	One-STrEP-Azurin-6xHis	Rabbit IgG	*Strep*-Tag II-Azurin-6xHis	One-STrEP-Azurin-6xHis	Rabbit IgG
S/B	2	120	947	86	113	954
EC_{50} (nM)	N/A	25.4	0.1	56	7.7	0.1



Donor bead	Streptavidin			*Strep*-Tactin®		
buffer	Universal Buffer	RPMI (10% FBS)	RPMI (1% FBS)	Universal Buffer	RPMI (10% FBS)	RPMI (1% FBS)
S/B	1560	142	165	1718	1203	1202
EC_{50} (nM)	0.7	0.6	0.6	0.9	1.0	1.1

Strep-Tactin® and Streptavidin-coated Alpha Donor beads were used in anti-GFP-conjugated AlphaLISA Acceptor bead evaluation assay performed in presence of RPMI media.

10 Summary

- Both *Strep*-Tactin® conjugated AlphaLISA Acceptor beads and Alpha Donor beads can serve for detection of either *Strep*®-tag II or One-STrEP tagged and/or biotin-labeled proteins.
- The performance of *Strep*-Tactin®-conjugated beads is not influenced by the presence of cell lysates as well as cell culture media. Furthermore, due to the lower affinity of *Strep*-Tactin® for free biotin compared to Streptavidin, the use of *Strep*-Tactin®-conjugated beads can improve the S/B of an AlphaLISA assay performed in biotin-containing buffers, as for example RPMI cell culture media.
- Depending on the assay components, the order of addition of Donor vs. Acceptor beads can influence the assay parameters like EC_{50} and S/B.
- *Strep*-Tactin®-conjugated beads can be used with success in complex assays like protein-protein interaction assays.

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