TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

PDGF-BB and PDGFRα (Human) Binding AlphaLISA Kit

Product No.: AL3064

Contents

Product Information

Application: This kit is designed for the detection of binding activity between Human PDGF-BB and PDGFRα, using a homogeneous AlphaLISA assay (no wash step). This assay can facilitate the design and development of antibody therapeutics by using competitive binding to PDGF-BB and PDGFRα.

IC_{50}: 0.7 µg/mL (average) using 10 nM:10 nM (PDGF-BB:PDGFRα) with human PDGF-BB antibody

Signal to background ratio: 214 (average) using 10 nM:10 nM (PDGF-BB:PDGFRα)

Kit contents: The kit contains 5 components: anti-6xHis AlphaLISA Acceptor beads, Streptavidin-coated Donor beads, Biotinylated human PDGF-BB, His tagged human PDGFRα and Casein Buffer (5X).

Storage: All kit components must be stored at 4 ˚C and in the dark.

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

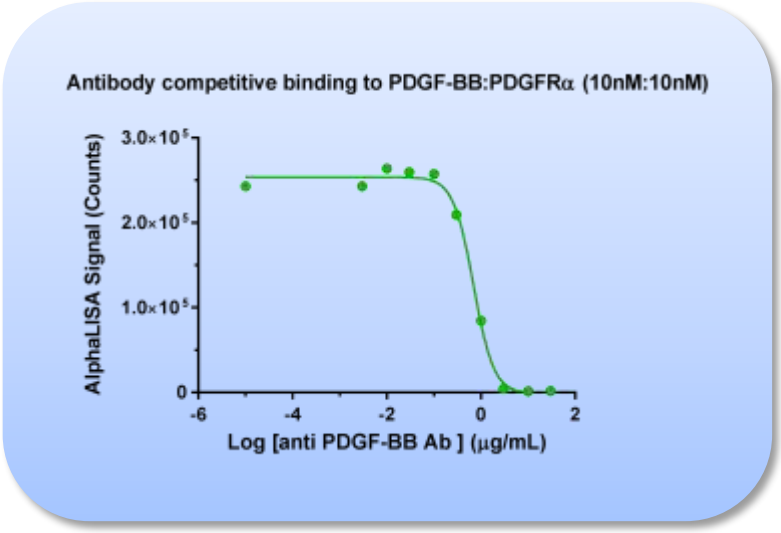


Figure 1. Typical inhibition curve in AlphaLISA 1X Casein Buffer. The data was generated using a Proxiplate™-384-SW microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals and the apparent binding dissociation constant IC_{50} were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on IC_{50} measurement.

PerkinElmer
For the Better

Analyte of Interest

Platelet-derived growth factor (PDGF) is one of numerous growth factors that regulate cell growth and division. PDGF is a dimeric glycoprotein that can be composed of two A subunits (PDGF-AA), two B subunits (PDGF-BB), or one of each (PDGF-AB). The dimeric isoforms are differentially expressed in various cell types and their effects are mediated through two cell surface tyrosine kinase receptors, platelet-derived growth factor receptors, termed alpha (PDGFRα) and beta (PDGFRβ). Differences exist in isoform binding to each receptor. PDGF and PDGFR are involved in human cancer development and progression through autocrine stimulation of tumor cell growth. Blocking PDGF and PDGFR binding has been considered as a potential target for antitumor and antiangiogenic therapy.

Description of the AlphaLISA Assay

AlphaLISA technology allows detecting the binding of target proteins in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated PDGF-BB binds to the Streptavidin-coated Alpha Donor beads, while His tagged PDGFRα is captured by Anti-His AlphaLISA Acceptor beads. When PDGF-BB binding to PDGFRα happens, Donor beads and Acceptor beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

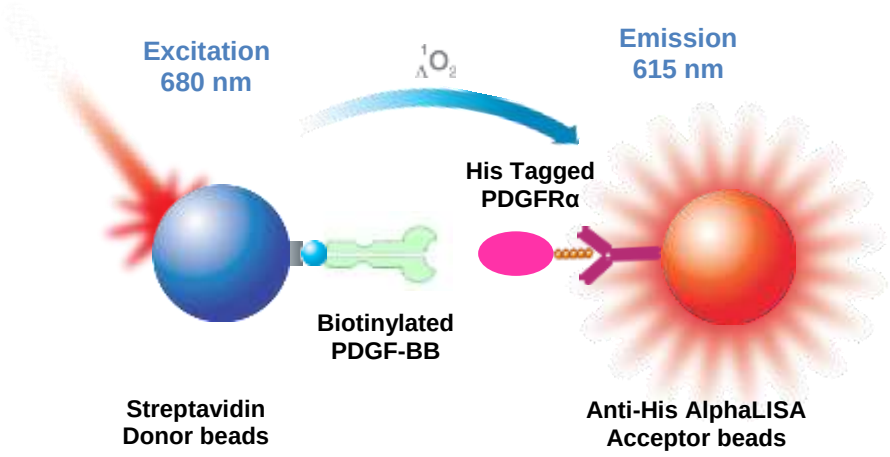


Figure 2. AlphaLISA Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.

Kit Content: Reagents and Materials

Kit components	AL C (500 assay points)**	AL F (5000 assay points)**
Anti-6xHis AlphaLISA Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	200 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	400 µL @ 5 mg/mL (1 brown tube, black cap)
Human PDGFRα (His Tagged)	2.89 µg (2 tubes, clear caps)	11.54 µg (5 tubes, clear caps)
Human PDGF-BB (Biotinylated)	1.23 µg (2 tubes, clear caps)	4.92 µg (5 tubes, clear caps)
Casein Buffer (5X)	10 mL, 1 small bottle	100 mL, 1 large bottle

* Extra buffer can be ordered separately (cat # AL014C: 10 mL, cat # AL014F: 100 mL).

** The number of assay points is based on an assay volume of 20 µL in 384 well plates using the kit components at the recommended concentrations.

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
EnVision®-Alpha Reader	PerkinElmer Inc.	-
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185

The following reagents might be required for particular applications:

Item	Supplier	Catalog number
Human PDGF-BB antibody	R&D Systems	AB-220-NA
Human PDGFRα antibody	R&D Systems	MAB322
Human PDGFRβ antibody	R&D Systems	AF385

PerkinElmer
For the Better

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes before use to improve recovery of content (2000g, 10-15 sec).
  Re-suspend the beads by vortexing before use. Do not vortex the proteins.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover the microplate with a lid or a plate sealing film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings for ProxiPlates (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.

Antibody Inhibitory Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an ***example*** for generating three inhibition curves in a 20 µL final assay volume (500 wells). If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

			Volume				
Format	**# of data points**	**Final**	**Inhibitor Or Antibody**	**His Tag PDGFRα**	**Biotinylated PDGF-BB**	**Mix of SA-Donor beads and anti His Acceptor beads**	**Plate recommendation**
AL C	250	40 µL	10 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290)
	500	20 µL	5 µL	5 µL	5 µL	5 µL	ProxiPlate™-384 SW (cat # 6008350) ProxiPlate™-384 HS (cat # 6008270)
	1 000	10 µL	2.5 µL	2.5 µL	2.5 µL	2.5 µL	ProxiPlate™-384 SW (cat # 6008350)

PerkinElmer
For the Better

AL F	5 000	20 µL	5 µL	5 µL	5 µL	5 µL	ProxiPlate™-384 SW (cat # 6008350)
	10 000	10 µL	2.5 µL	2.5 µL	2.5 µL	2.5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Protocol for anti PDGF-BB antibody inhibition of PDGF-BB and PDGFRα Binding AlphaLISA Assay

1) Preparation of 1X Casein Buffer

   a. Add 2 mL of Casein buffer (5X) to 8 mL water to make 10mL of 1X Casein Buffer.

2) Preparation of 4X anti PDGF-BB antibody:

   a. Reconstitute 100 µg of human PDGF-BB antibody in 100 µL PBS.
   b. Measure human PDGF-BB antibody concentration with NanoDrop and dilute it to 0.5 mg/mL.
   c. Prepare standard dilutions as follows in 1X Casein Buffer (change tip between each standard dilution):

Tube	Vol. of Antibody	Vol. of diluent (µL) *	[Ab] in standard curve	
			4 X (g/mL)	1X (µg/mL)
A	12 µL of 0.5 mg/mL antibody	38	1.2E-04	30
B	15 µL of tube A	35	4.0E-05	10
C	15 µL of tube B	30	1.2E-05	3
D	15 µL of tube C	35	4.0E-06	1
E	15 µL of tube D	30	1.2E-06	0.3
F	15 µL of tube E	35	4.0E-07	0.1
G	15 µL of tube F	30	1.2E-07	0.03
H	15 µL of tube G	35	4.0E-08	0.01
I	15 µL of tube H	30	1.2E-08	0.003
J	15 µL of tube I	35	4.0E-09	0.001
K	15 µL of tube J	30	1.2E-09	0.0003
M (background)	0	50	0	0

* Dilute standards in diluent (e.g. 1X Casein Buffer).
At low concentrations of the protein, a significant amount of the protein can bind to the vial. Therefore, load the standard dilutions in the assay microplate within 60 minutes of preparation.

3) Preparation of 4X biotinylated human PDGF-BB (40 nM):

For 500 points kit (AL3064C):

   a. Reconstitute both biotinylated PDGF-BB (1.23 µg) tubes in 100 µL water each to make 500 nM concentration
   b. Add 200 µL of 500 nM biotinylated PDGF-BB to 2300 µL of 1X Casein Buffer.
   c. Prepare just before use.

PerkinElmer
For the Better

For 5000 points kit (AL3064F):

a. Reconstitute biotinylated PDGF-BB (4.92 µg) in 400 µL water to make 500 nM concentration
b. Add 200 µL of 500 nM biotinylated PDGF-BB to 2300 µL of 1X Casein Buffer.
c. Prepare just before use.

4) Preparation of 4X human His -PDGFRα (40 nM)

For 500 points kit (AL3064C):

a. Reconstitute both His-PDGFRα (2.89 µg) in 100 µL water each to make 500 nM concentration
b. Add 200 µL of 500 nM of His PDGFRα into 2300 µL 1X Casein Buffer.
c. Prepare just before use.

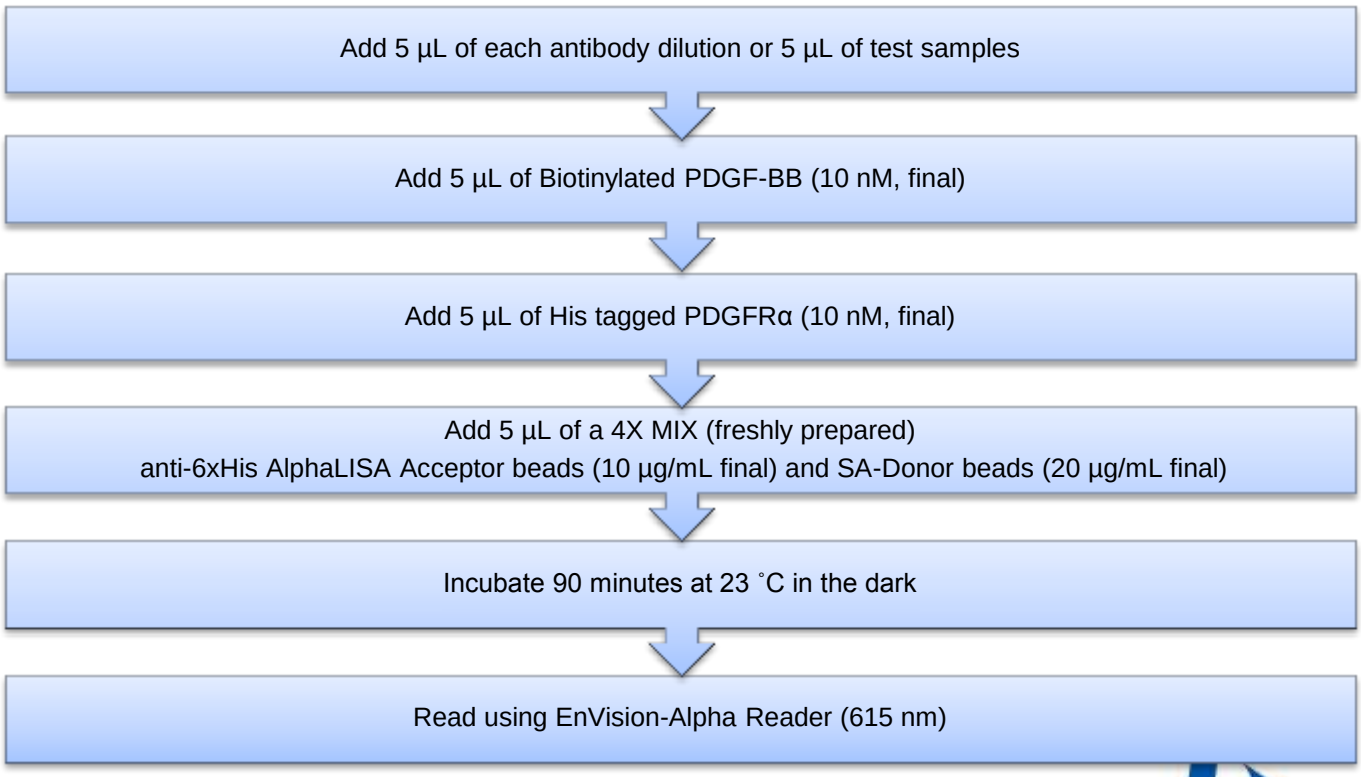
For 5000 points kit (AL3064F):

a. Reconstitute His-PDGFRα (11.54 µg) in 400 µL water to make 500 nM concentration
b. Add 200 µL of 500 nM of His PDGFRα into 2300 µL 1X Casein Buffer.
c. Prepare just before use.

5) Preparation of the mix of 4X AlphaLISA anti-His Acceptor beads (40 µg/mL) and 4X Streptavidin (SA) Donor beads (80 µg/mL):

a. Keep the beads under subdued laboratory lighting.
b. Add 20 µL of 5 mg/mL AlphaLISA Anti-His Acceptor beads and 40 µL of 5 mg/mL SA-Donor beads to 2440 µL of 1X Casein Buffer.
c. Prepare just before use.

6) In a ProxiPlate (384 wells)



PerkinElmer
For the Better

Typical Inhibition Data:

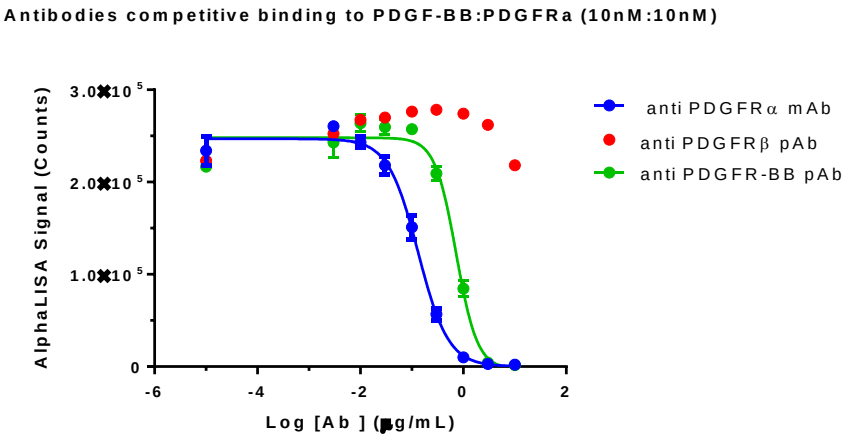


Figure 3. Antibodies blocking binding of PDGF-BB to PDGFRα. The anti-PDGFRβ antibody showed as a negative control (red points). The IC_{50} values are 0.13 and 0.73 µg/mL for anti-PDGFRα monoclonal antibody (blue points) and anti-PDGF-BB polyclonal antibody (green points) respectively and were calculated by using nonlinear regression fitting with GraphPad Prism 6. The data was generated using a ProxiPlate-384-SW microplate and an EnVision-Alpha Reader 2103 with alpha option.

PerkinElmer
For the Better

Troubleshooting Guide

You will find below recommendations for common situations that you might encounter with your AlphaLISA binding assay. If further assistance is needed, do not hesitate to contact our technical support team for assistance.

Issue	Recommendations and Comments
High background signal	• Incubation time is longer than recommended range.
Low AlphaLISA signal	• Optimize EnVision with ProxiPlate format.
High variation between replicates or low Z' values	• Make sure that reagents are at the bottom of the well by tapping or swirling the plate gently on a smooth surface after each addition.

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/alphalisa-alphascreen-no-wash-assays/alpha-troubleshooting.html

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

This product is not for resale or distribution except by authorized distributors.

LIMITED WARRANTY: PerkinElmer warrants that, at the time of shipment, the above named product is free from defects in material and workmanship and conforms to the specifications set forth above. PerkinElmer makes no other warranty, express or implied with respect to the product and expressly disclaims any warranty of merchantability or fitness for any particular purpose. Notification of any breach of the foregoing warranty must be made within 60 days of receipt of the product, unless otherwise provided in writing by PerkinElmer. No claim shall be honored if the customer fails to notify PerkinElmer within the period specified. The sole and exclusive remedy of the customer for any breach of the foregoing warranty is limited to either the replacement of the non-conforming product or the refund of the invoice price of the product. PERKINELMER SHALL NOT BE LIABLE FOR ANY DIRECT, INDIRECT, SPECIAL, INCIDENTAL, CONSEQUENTIAL OR PUNITIVE DAMAGES, WHETHER BASED ON CONTRACT, TORT, STRICT LIABILITY OR OTHERWISE, ARISING OUT OF THE DESIGN, MANUFACTURE, SALE, DELIVERY, OR USE OF THE PRODUCTS, EVEN IF THE LIMITED REMEDIES PROVIDED HEREIN FAIL OF THEIR ESSENTIAL PURPOSE OR PERKINELMER IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright© 2012, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.