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PDGF-BB and PDGFR β (Human) Binding AlphaLISA Kit

Product No.: AL3065

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Product Information

Application: This kit is designed for the detection of binding activity between Human PDGF-BB and PDGFR β , 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 PDGFR β .

IC₅₀: 0.3 μ g/mL (average) using 5 nM:1.5 nM (PDGF-BB:PDGFR β) with human PDGF-BB antibody

Signal to background ratio: 303 (average) using 5 nM:1.5 nM (PDGF-BB:PDGFR β)

Kit contents: The kit contains 5 components: anti-6xHis AlphaLISA Acceptor beads, Streptavidin-coated Donor beads, Biotinylated human PDGF-BB, His tagged human PDGFR β 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.

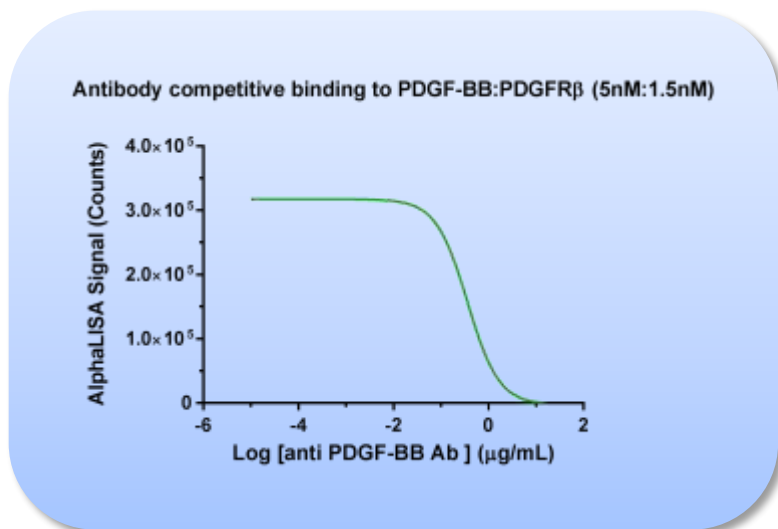


Figure 1. Typical inhibition curve in AlphaLISA 1X Casein Buffer. The data was generated using a ProxiplateTM-384-SW microplate and the EnVision[®] Multilabel Plate Reader 2103 with Alpha option.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals and the apparent binding dissociation constant IC₅₀ 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 IC₅₀ measurement.

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 (PDGFR α) and beta (PDGFR β). 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 PDGFR β is captured by Anti-His AlphaLISA Acceptor beads. When PDGF-BB binding to PDGFR β 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).

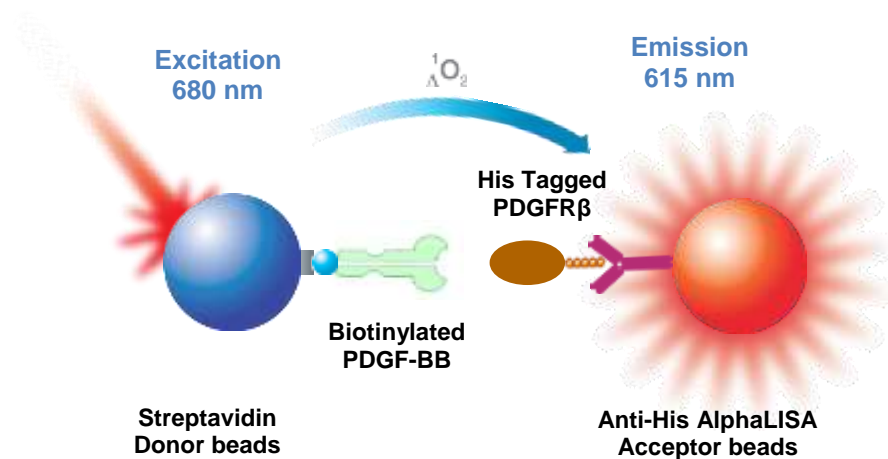


Figure 2. AlphaLISA Assay Principle.

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	AL3065C (500 assay points)**	AL3065F (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, <u>white</u> cap)	200 µL @ 5 mg/mL (1 brown tube, <u>white</u> 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, <u>black</u> cap)	400 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)
Human PDGFRβ (His Tagged)	4.2 µg (1 tube, <u>clear</u> cap)	4.2 µg (3 tubes, <u>clear</u> caps)
Human PDGF-BB (Biotinylated)	1.23 µg (1 tube, <u>clear</u> cap)	4.92 µg (3 tubes, <u>clear</u> 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

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 μ L 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.

Format	# of data points	Final	Volume				Plate recommendation
			Inhibitor Or Antibody	His Tag PDGFR β	Biotinylated PDGF-BB	Mix of SA-Donor beads and anti His Acceptor beads	
AL306 5C	250	40 μ L	10 μ L	10 μ L	10 μ L	10 μ L	White $\frac{1}{2}$ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290)
	500	20 μ L	5 μ L	5 μ L	5 μ L	5 μ L	ProxiPlate™-384 SW (cat # 6008350) ProxiPlate™-384 HS (cat # 6008270)
	1 000	10 μ L	2.5 μ L	2.5 μ L	2.5 μ L	2.5 μ L	ProxiPlate™-384 SW (cat # 6008350)

AL306 5F	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 PDGFR β 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 (20 nM):

For 500 points kit (AL3065C):

- a. Reconstitute biotinylated PDGF-BB (1.23 µg) in 100 µL water to make 500 nM concentration
- b. Add 100 µL of 500 nM biotinylated PDGF-BB to 2400 µL of 1X Casein Buffer.
- c. Prepare just before use.

For 5000 points kit (AL3065F):

- a. Reconstitute biotinylated PDGF-BB (4.92 µg) in 400 µL water to make 500 nM concentration
- b. Add 100 µL of 500 nM biotinylated PDGF-BB to 2400 µL of 1X Casein Buffer.
- c. Prepare just before use.

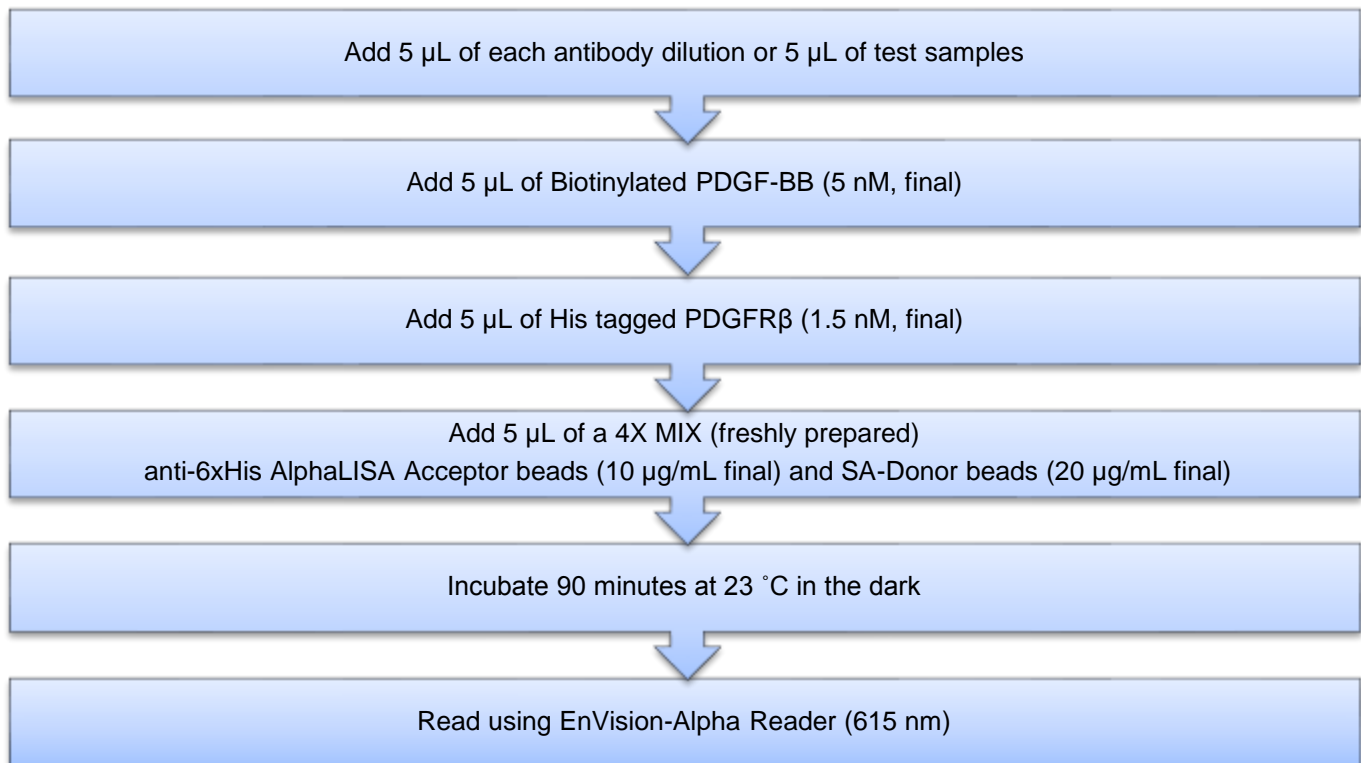
4) Preparation of 4X human His -PDGFRβ (6 nM)

- a. Reconstitute His-PDGFRβ (4.2 µg) in 100 µL water to make 500 nM concentration
- b. Add 30 µL of 500 nM of His PDGFRβ into 2470 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)



Typical Inhibition Data:

Antibodies competitive binding to PDGF-BB-biotin:PDGFR β (5nM:1.5nM)

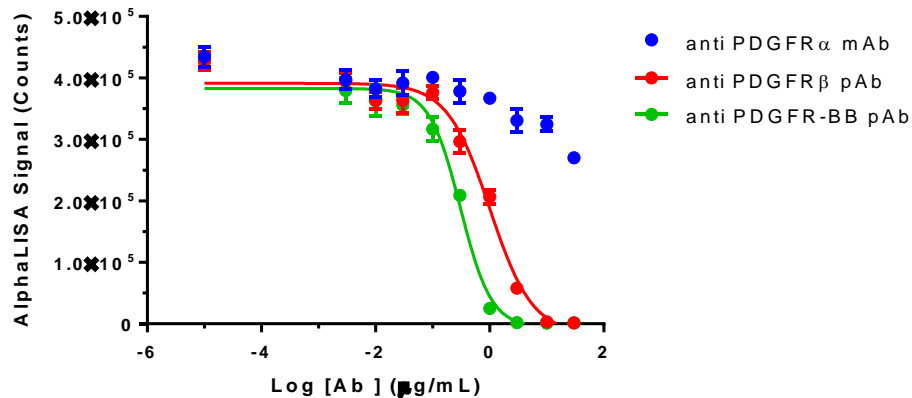


Figure 3. Antibodies blocking binding of PDGF-BB to PDGFR β . The anti-PDGFR α antibody showed as a negative control (blue points). The IC₅₀ values are 1.0 and 0.3 µg/mL for anti-PDGFR β polyclonal antibody (red points) and anti-PDGFR-BB polyclonal antibody (green points) respectively and were calculated 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.

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	<ul style="list-style-type: none">Incubation time is longer than recommended range.
Low AlphaLISA signal	<ul style="list-style-type: none">Optimize EnVision with ProxiPlate format.
High variation between replicates or low Z' values	<ul style="list-style-type: none">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>

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