

Research Use Only. Not for use in diagnostic procedures.

## AlphaLISA Human Cereblon Binding Kit

Product No.: AL3147 C/F

### Contents

	Page
Product Information.....	2
Quality Control.....	2
Analyte of Interest.....	3
Description of the AlphaLISA Assay .....	3
Precautions.....	3
Kit content: Reagents and Materials.....	4
Recommendations.....	5
Competition Assay Procedure.....	5
Troubleshooting Guide.....	8

## Product Information

- Application:** This kit is designed to assess orthosteric binders of CRBN protein using a homogeneous no wash AlphaLISA binding assay.
- Sensitivity:**  $IC_{50}$ : 2.2 $\mu$ M (average, using AlphaLISA CRBN Binding kit Standard (Lenalidomide)). To calculate binding affinity (Ki) with the Cheng-Prusoff equation, use  $K_{d\text{ligand}}$  of 15nM.
- Signal to background ratio:** 234 (average) using 3 nM GST-CRBN protein and 20 nM biotinylated ligand
- Kit contents:** The kit contains 6 components: Glutathione AlphaLISA Acceptor beads, Streptavidin-coated Donor beads, Biotinylated CRBN ligand, GST tagged CRBN protein, AlphaLISA CRBN Binding kit Standard (Lenalidomide) and AlphaLISA PPI buffer.
- Storage:** The kit components must be stored at 4 °C in the dark. Reconstituted GST-tagged CRBN protein can be stored at -80°C and AlphaLISA CRBN Binding kit Standard (Lenalidomide) at -20°C for 14 days. Reconstituted biotinylated CRBN ligand can be stored at -80°C for 14 days. S/B may highly decrease due to a storage at -20°C, or a longer-term storage of the biotinylated CRBN ligand or multiple freeze-thaw cycles.
- Stability:** This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging (lyophilized) and the recommended storage conditions (+4°C).

## Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals 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 and minimum counts may vary between bead lots and the instrument used.

## Analyte of Interest

Cereblon, also known as CRBN, is involved in many biological processes and is closely associated with proliferation, apoptosis, cell metabolism, and the occurrence of many diseases. Cereblon is also one of the two most popular E3 ligases which is recruited by bifunctional Proteolysis-targeting chimeras (PROTACs) to induce ubiquitination and subsequent proteasomal degradation of a targeted protein. Cereblon interacts with several proteins to form the functional E3 ubiquitin ligase complex, in which CRBN functions as a substrate receptor of the E3 ubiquitin ligase complex and targets proteins for proteolysis.

Identifying new Cereblon ligands is therefore a way to control biological processes involved in oncology, neurodegenerative, or metabolic diseases by inducing the proteasomal-dependent degradation of undesired proteins.

## Description of the AlphaLISA Assay

The AlphaLISA detection of CRBN binding uses Glutathione AlphaLISA acceptor beads to capture the GST-tagged CRBN protein and Streptavidin-coated donor beads to capture the biotinylated ligand. Donor beads and acceptor beads come into proximity through ligand binding to CRBN protein. Excitation of the Donor beads provokes the release of singlet oxygen that triggers a cascade of energy transfer reactions in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 1).

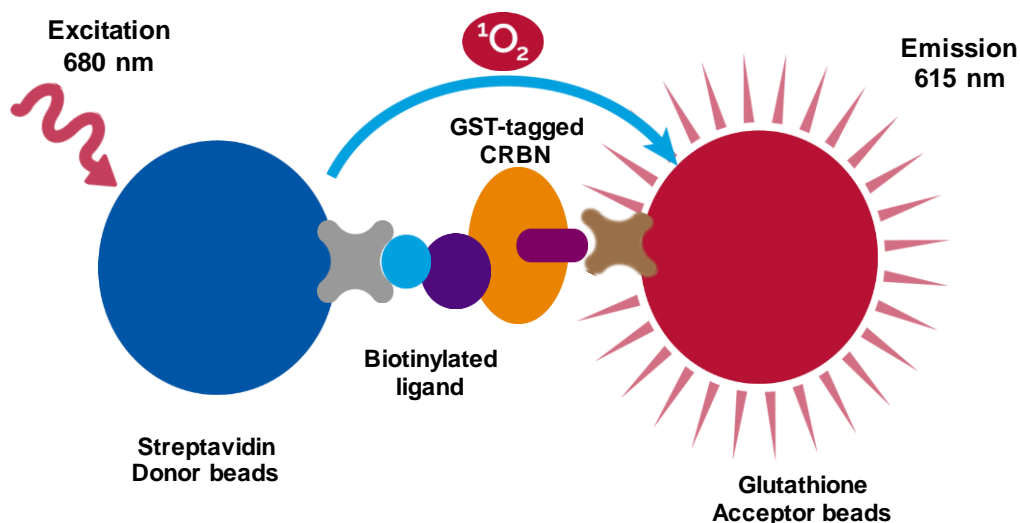


Figure 1. AlphaLISA Assay Principle.

## 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.
- All blood components and biological materials should be handled as potentially hazardous.

## Kit Content: Reagents and Materials

Kit components	AL3147C*** (500 assay points)	AL3147F*** (5000 assay points)
Glutathione AlphaLISA Acceptor beads stored in 50 mM Tris pH 8.0, 150 mM NaCl, 0.1% Tween-20, 0.05% Kathon	40 $\mu$ L @ 5 mg/mL (1 brown tube, <u>white</u> cap)	400 $\mu$ L @ 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% Kathon, pH 7.4	40 $\mu$ L @ 5 mg/mL (1 brown tube, <u>black</u> cap)	400 $\mu$ L @ 5 mg/mL (1 brown tube, <u>black</u> cap)

Ligand (Biotinylated)*	275 ng lyophilized (1 tube, <u>clear</u> cap)	275 ng lyophilized (10 tubes, <u>clear</u> cap)
CRBN protein (GST tagged)*	2.3 µg lyophilized (1 tube, <u>clear</u> cap)	2.3 µg lyophilized (10 tubes, <u>clear</u> cap)
AlphaLISA CRBN Binding kit Standard (Lenalidomide) *	1.87mg, lyophilized (1 tube, <u>clear</u> cap)	1.87mg, lyophilized (2 tubes, <u>clear</u> cap)
AlphaLISA PPI Buffer (5X)**	10 mL, 1 small bottle	100 mL, 1 large bottle

\* Reconstitute CRBN protein and ligand in 100 µL Milli-Q® grade H<sub>2</sub>O respectively. Reconstitute AlphaLISA CRBN Binding kit Standard (Lenalidomide) in 100µL DMSO. The reconstituted reagents should be used within 60 minutes.

\*\* Extra buffer can be ordered separately (cat # AL015C: 10 mL, cat # AL015F: 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.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.

#### Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	PerkinElmer Inc.	6050185
AlphaPlate-384, Shallow Well (ProxiPlate)	PerkinElmer Inc.	6008350 6008359
EnVision®-Alpha Reader	PerkinElmer Inc.	-

The following reagents might be required for particular applications:

Item	Supplier	Catalog number
AlphaLISA CRBN Binding kit Standard (Lenalidomide)	PerkinElmer Inc.	AL3147S
Pomalidomide	Tocris	6302
dBRD9	Tocris	6606
VH032	Tocris	6462

## 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 (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend the beads by vortexing before use. Do not vortex the proteins.
- Use Milli-Q® grade H<sub>2</sub>O to dilute 5X AlphaLISA PPI Buffer and to reconstitute the lyophilized reagents excepted the AlphaLISA CRBN Binding kit Standard (Lenalidomide). Use DMSO to reconstitute the lyophilized AlphaLISA CRBN Binding kit Standard (Lenalidomide).
- 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 microplates with TopSeal™-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (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.

## Competition Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an **example** for generating 288 assay points in a 20 µL final assay volume per point. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes. If a different number of samples are tested, the volumes of all reagents must be adjusted accordingly.
- The dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

### One Incubation Step Protocol described as below:

#### 1) Preparation of 1X AlphaLISA PPI Buffer (for 10 mL):

Add 2 mL of 5X AlphaLISA PPI Buffer to 8 mL H<sub>2</sub>O.

#### 2) Preparation of serial dilution of 4X AlphaLISA CRBN Binding kit Standard (Lenalidomide):

- a. Reconstitute lyophilized AlphaLISA CRBN Binding kit Standard (1,87 mg) in 100 µL DMSO to make a 72mM stock solution of AlphaLISA CRBN Binding kit Standard.
- b. Prepare serial dilutions of 4X AlphaLISA CRBN Binding kit Standard in 1x AlphaLISA PPI buffer as mentioned in the table below, (do not forget to change tips between each dilution):

Tube	Volume of standard	Volume of 1X buffer	[Standard] ( $\mu\text{M}$ ) (4X)	[Standard] ( $\mu\text{M}$ ) (1X)
A	10 $\mu\text{L}$ of 72mM stock solution	170 $\mu\text{L}$	4000	1000
B	60 $\mu\text{L}$ of tube A	140 $\mu\text{L}$	1 200	300
C	60 $\mu\text{L}$ of tube B	120 $\mu\text{L}$	400	100
D	60 $\mu\text{L}$ of tube C	140 $\mu\text{L}$	120	30
E	60 $\mu\text{L}$ of tube D	120 $\mu\text{L}$	40	10
F	60 $\mu\text{L}$ of tube E	140 $\mu\text{L}$	12	3
G	60 $\mu\text{L}$ of tube F	120 $\mu\text{L}$	4	1
H	60 $\mu\text{L}$ of tube G	140 $\mu\text{L}$	1	0.3
I	60 $\mu\text{L}$ of tube H	120 $\mu\text{L}$	0.4	0.10
J	60 $\mu\text{L}$ of tube I	140 $\mu\text{L}$	0.12	0.03
K	60 $\mu\text{L}$ of tube J	120 $\mu\text{L}$	0.04	0.01
L	0	140 $\mu\text{L}$	0	0

3. Preparation of 4X GST tagged CRBN protein:

- a. Reconstitute lyophilized GST-CRBN protein in 100  $\mu\text{L}$  H<sub>2</sub>O to make a 300 nM GST-CRBN protein stock solution.
- b. Add 10  $\mu\text{L}$  of the 300nM GST-CRBN protein stock solution to 240  $\mu\text{L}$  of 1X AlphaLISA PPI buffer to obtain a 12nM working solution of GST-CRBN protein.

Prepare just before use.

4. Preparation of 4X biotinylated ligand:

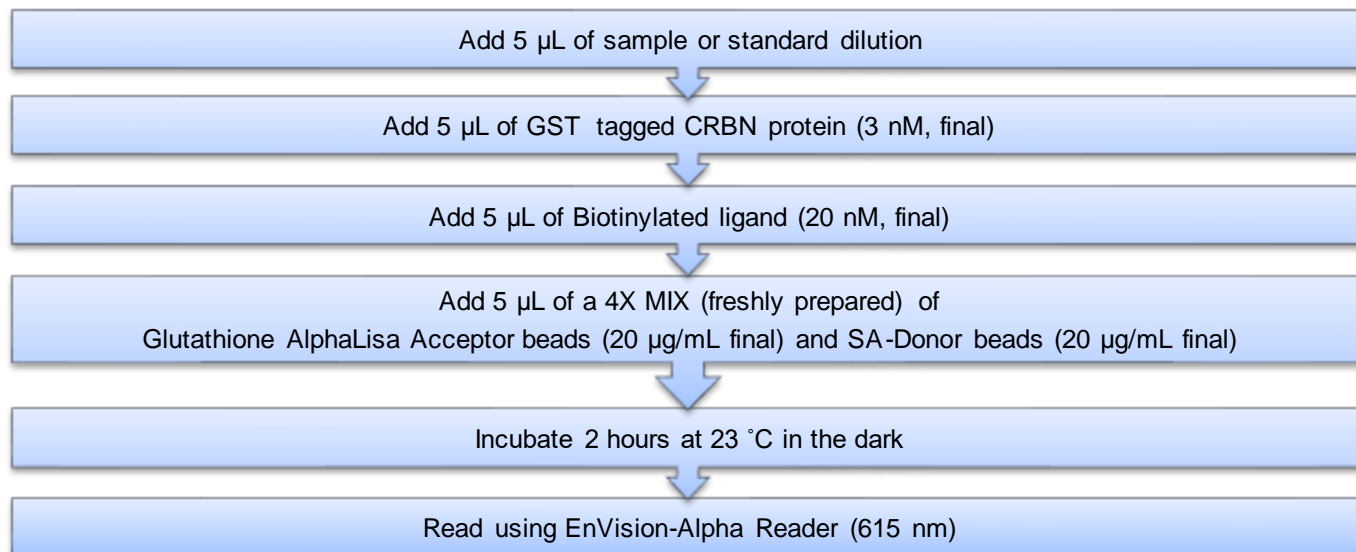
- a. Reconstitute lyophilized biotinylated ligand in 100  $\mu\text{L}$  H<sub>2</sub>O to make a 2000 nM stock solution.
- b. Add 10  $\mu\text{L}$  of 2000 nM biotinylated ligand to 240  $\mu\text{L}$  1X AlphaLISA PPI buffer to obtain a 80nM stock solution of biotinylated ligand.

Prepare just before use.

5. Preparation of the mix of 4X Glutathione AlphaLISA Acceptor beads (20  $\mu\text{g}/\text{mL}$ ) and 4X Streptavidin (SA) Donor beads (20  $\mu\text{g}/\text{mL}$ ):

- a. Keep the beads under subdued laboratory lighting.
- b. Add 4  $\mu\text{L}$  of 5 mg/mL Glutathione AlphaLISA Acceptor beads and 4  $\mu\text{L}$  of 5 mg/mL SA-Donor beads to 242  $\mu\text{L}$  of 1X AlphaLISA PPI buffer
- c. Prepare just before use.

6. Distribute the prepared reagents in a shallow well AlphaPlate (384 wells):



**Read Settings:** AlphaLISA signal is detected using an EnVision Multilabel Reader equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser: 680 nm, Excitation Time: 180 ms, Mirror: 640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).

#### Typical competitive binding Data:

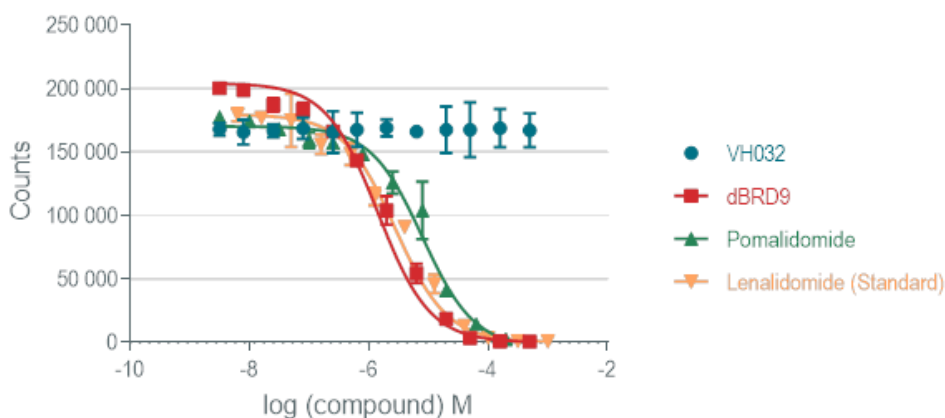


Figure 2. Illustrations with Lenalidomide Standard, Pomalidomide and dBRD9 as competitors, competitively bind to CRBN with  $K_i = 1.5\mu\text{M}$ ,  $3.2\mu\text{M}$  and  $0.6\mu\text{M}$  respectively. VH032 was measured as negative control. All pharmacological values (IC<sub>50</sub>,  $K_i$ ) were calculated by using a nonlinear regression fitting with GraphPad Prism 9.

#### 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"><li>• Buffer is not freshly made. Make new.</li><li>• Incubation time is longer than recommended range.</li></ul>
Low AlphaLISA signal	<ul style="list-style-type: none"><li>• Optimize EnVision with Plate format.</li></ul>
High variation between replicates or low Z' values	<ul style="list-style-type: none"><li>• 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.</li></ul>

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

<https://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/alphalisa-phascreen-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](http://www.perkinelmer.com)

For a complete listing of our global offices, visit [www.perkinelmer.com/ContactUs](http://www.perkinelmer.com/ContactUs)

Copyright © 2019, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.