SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
PROTAC Optimization Kit for CDK Kinase-Cereblon Binding

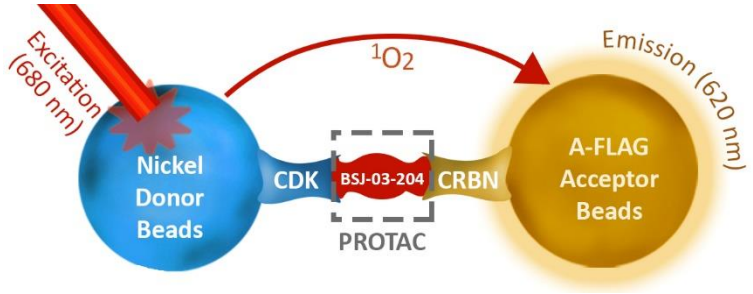
Catalog #79924
Size: 384 reactions

DESCRIPTION: The *PROTAC Optimization Kit for CDK Kinase-Cereblon Binding* is designed for testing and profiling of PROTACs directed against the CDK Kinase family and Cereblon. Cereblon (CRBN) is a Substrate recognition component of the DCX (DDB1-CUL44-Rbx1) E3 protein ligase complex that mediates the ubiquitination and subsequent proteasomal degradation of target proteins.

The *PROTAC Optimization Kit for CDK Kinase-Cereblon Binding* comes in a convenient AlphaLISA® format, with BSJ-03-204 PROTAC, CDK PROTAC buffer, purified CDK4 and CDK6, and CRBN for 384 reactions. The CDK inhibitor Palbociclib is included as a control inhibitor of PROTAC binding to CDK. With this kit, only three simple steps on a microtiter plate are required for PROTAC activity detection. First, a sample containing PROTAC is incubated with CRBN and CDK4 or CDK6 Kinase. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100255	Cereblon	2x10 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
40104	CDK4/Cyclin D3	25 µg	-80°C	
40206	CDK6/Cyclin D3	10 µg	-80°C	
	BSJ-03-204 (200 µM)	2x20 µl	-80°C	
	3x CDK PROTAC Buffer	4 ml	-20°C	
	Palbociclib (MW=574)	120 µg	-20°C	



6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-FLAG acceptor beads, 5 mg/ml (PerkinElmer #AL112C)
Alpha Nickel donor beads, 5 mg/ml (PerkinElmer #AS101D)
Optiplate 384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for identifying and optimizing PROTACs targeting the CDK family of kinases, the design of novel molecules targeting CRBN, and comparison of activities of different PROTACs.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of culture medium RPMI 1640 at >1% leads to signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least six months from date of receipt when stored as directed.

REFERENCE: Brand, M., *et al. Cell Chem. Biol.* 2019; **26(2):**300-306.

SAFETY WARNING: BSJ-03-204 is a thalidomide-derivative, which is known to cause severe birth defects in humans**. It is very important to use all appropriate precautions when handling this compound.**

ASSAY PROTOCOL 1 -- Optimization of Bromodomain-Cereblon Binding

This protocol is designed to test the binding affinity of various PROTAC samples to the CDK kinases or cereblon complex.

All samples and controls should be tested in duplicate. All incubations are performed with slow shaking on a rotator platform.

Step 1:
1) Prepare 1x CDK PROTAC buffer by adding 1 part of 3x CDK PROTAC buffer plus 2 parts of distilled water. Prepare only amount needed for the experiment. Aliquot the remaining undiluted 3x CDK PROTAC buffer and store at -20°C.

2) PROTAC dilution: Add 980 ∝l of 1x CDK PROTAC buffer to 20 µl of BSJ-03-204 (200 µM)to make a 4 µM solution. Note: final concentration of BSJ-03-204 in the assay may be in the range 0.5-1 µM.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

3) Thaw Cereblon and CDK on ice. Upon first thaw, briefly spin tubes containing proteins to recover full content of the tubes. Aliquot proteins into single use aliquots. Store remaining undiluted proteins in aliquots at -80°C immediately. Note: Both CDK and Cereblon are sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

4) Dilute Cereblon in 1X CDK PROTAC buffer at 20 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

5) Dilute CDK4 in 1X CDK PROTAC buffer at 50 ng/µl. Alternatively, dilute CDK6 at 28 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

6) Prepare master mix: N wells × (2.5 µl Cereblon (25 ng/µl) + 2.5 µl CDK + 2.5 µl 1x CDK PROTAC buffer. Add 7.5 µl of master mixture to every well.

Reagent	Blank	Positive Control	Test PROTAC
Cereblon (20 ng/µl)	2.5 µl	2.5 µl	2.5 µl
CDK (50 ng/µl) or CDK6 (28 ng/µl)	2.5 µl	2.5 µl	2.5 µl
1x CDK PROTAC buffer	5.0 µl	2.5 µl	2.5 µl
Test PROTAC	–	–	2.5 µl
BSJ-03-204 (4 µM)	–	2.5 µl	–
Total	10 µl	10 µl	10 µl

7) For the wells labeled as "Blank", add 2.5 µl 1x CDK PROTAC buffer. Dilute Test PROTAC in 1x CDK PROTAC buffer. Add 2.5 µl of diluted Test PROTAC to each well designated “Test PROTAC”. Add 2.5 µl of diluted BSJ-03-204 to each well designated “Positive Control”.

8) Incubate at room temperature for one hour.

Note: Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:
Dilute anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x CDK PROTAC buffer. Add 10 µl per well. Shake on a rotator platform for 60 minutes at room temperature.

Step 3:
1) Dilute Nickel donor beads (PerkinElmer #AS101D) 125-fold with 1x CDK PROTAC buffer. Add 10 µl per well. Shake on a rotator platform for 30-60 minutes at room temperature.

2) Read Alpha-counts. “Blank” value should be subtracted from all readings.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

ASSAY PROTOCOL 2 -- Competitive Inhibition of the PROTAC

This protocol is designed to measure inhibition of the PROTAC binding to the CDK kinases. The protocol can be easily modified to study inhibitors of the PROTAC to the cereblon complex.

All samples should be tested in duplicate. All incubations are performed with slow shaking on a rotator platform.

Step 1:

1) Prepare 1x CDK PROTAC buffer as in Protocol 1.

2) Dilute BSJ-03-204 as in Protocol 1.

3) Thaw Cereblon and CDK on ice. Upon first thaw, briefly spin tubes containing proteins to recover full content of the tubes. Aliquot proteins into single use aliquots. Store remaining undiluted proteins in aliquots at -80°C immediately. *Note: Both CDK and Cereblon are sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute Cereblon in 1X CDK PROTAC buffer at 20 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

5) Dilute CDK4 in 1X CDK PROTAC buffer at 50 ng/µl. Alternatively, dilute CDK6 at 28 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

6) Prepare master mix: N wells × (2.5 µl Cereblon (5 ng/µl) + 2.5 µl CDK4 or CDK6. Add 5 µl of master mixture to every well.

7) For the wells labeled as "Blank", add 2.5 µl 1x CDK PROTAC buffer.

8) Add 2.5 µl of test compound solution to each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 2.5 µl of the same solution without the test compound (“Compound buffer”). We recommend using 1x CDK PROTAC buffer with proper concentration of DMSO as Compound buffer. Preincubate the test compound with the cereblon and CDK for up to 30 minutes at room temperature at slow shaking.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Reagent	Blank	Positive Control	Inhibitor Control	Test Inhibitor
1x CDK PROTAC buffer	2.5 µl	–	–	–
Cereblon (20 ng/µl)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
CDK4 (50 ng/µl) or CDK6 (28 ng/µl)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Test Compound	–	–	–	2.5 µl
Compound buffer*	2.5 µl	2.5 µl	–	–
Palbociclib (diluted)	–	–	2.5 µl	–
BSJ-03-204 (4 µM)	–	2.5 µl	2.5 µl	2.5 µl
Total	10 µl	10 µl	10 µl	10 µl

*Typically, 1x CDK PROTAC buffer with proper concentration of DMSO.

9) Palbociclib dilution: Resuspend tube with Palbociclib with 1000 µl of 1x CDK PROTAC buffer to make a 200 µM solution. For the wells labeled as "Inhibitor Control", add 2.5 µl diluted Palbociclib.

10) Initiate reaction by adding 2.5 µl of diluted BSJ-03-204 prepared as described in Protocol 1 above to wells labeled “Positive Control", “Inhibitor Control” and "Test Inhibitor". Incubate at room temperature for one hour.

Note: Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Dilute anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x CDK PROTAC buffer. Add 10 µl per well. Shake on a rotator platform for 60 minutes at room temperature.

Step 3:

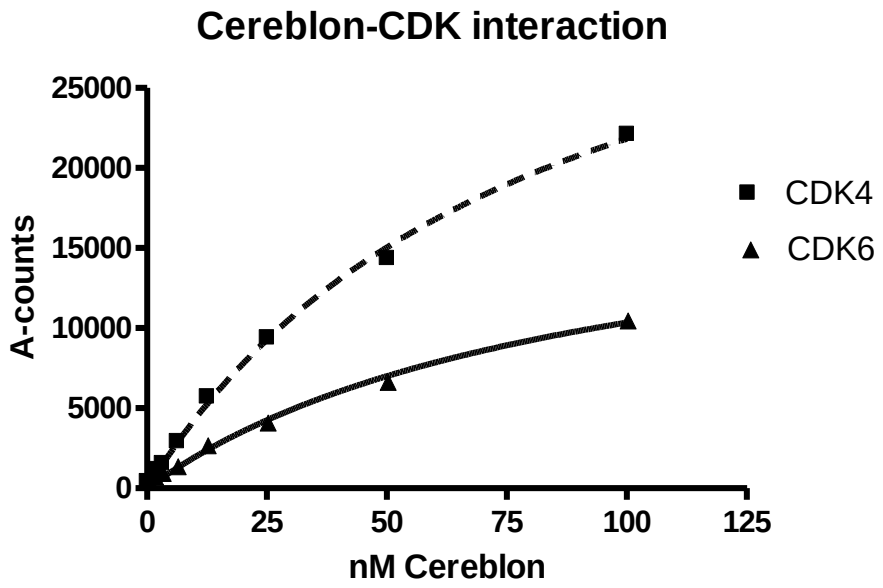
1) Dilute Nickel donor beads (PerkinElmer #AS101D) 125-fold with 1x CDK PROTAC buffer. Add 10 µl per well. Shake on a rotator platform for 30-60 minutes at room temperature.

2) Read Alpha-counts. “Blank” value should be subtracted from all readings.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Examples of Assay Results:

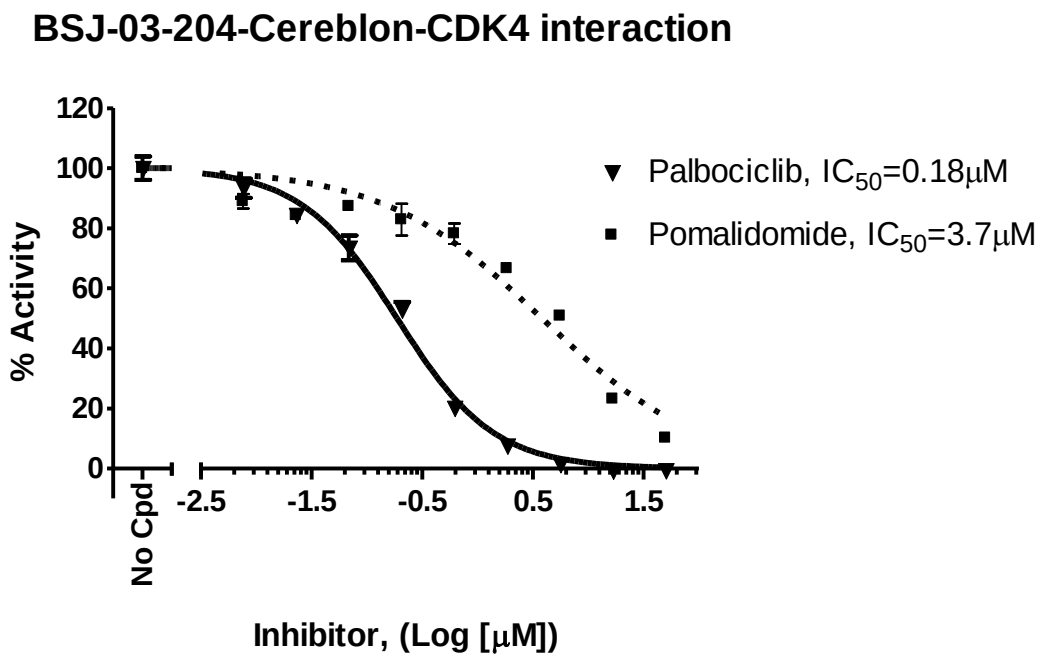
Experiment 1. Titration of CRBN at fixed concentration of CDK.



BSJ-03-204-mediated interaction of Cereblon with CDK, measured using the *PROTAC Optimization Kit for CDK Kinase-Cereblon Binding*, BPS Bioscience #79924. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Experiment 2. Effect of CDK Kinase or CRBN inhibitors.



Inhibition by Palbociclib or Pomalidomide of BSJ-03-204-mediated interaction of Cereblon with CDK4, measured using the *PROTAC Optimization Kit for CDK Kinase-Cereblon Binding*, BPS Bioscience #79924. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	**Cat. #**	**Size**
CDK4/Cyclin D3	40104	10 µg
CDK6/Cyclin D3	40206	20 µg
CDK6/Cyclin D1	40097	10 µg
CDK4(EE,T172A)/Cyclin D1, His-tag	40094	20 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex	100329-1	10 µg
ELOB/ELOC/VHL Complex	100361-1	10 µg
VHL/CUL2/ELOB/ELOC/RBX1 Complex	100373-1	10 µg
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 rxns.
Cereblon Binding Assay Kit	79899	96 rxns.
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 rxns.