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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

c-CBL TR-FRET Assay Kit

Catalog # 79786
Size: 384 reactions

DESCRIPTION: Casitas B lineage lymphoma (c-Cbl) is a multifunctional protein with ubiquitin E3 ligase activity capable of degrading diverse sets of proteins. c-Cbl is a ubiquitously expressed mammalian protein that plays a vital role in fundamental cellular functions including cell survival, migration, and proliferation. Recent evidence suggests a critical role for c-Cbl in angiogenesis and human solid organ tumors, making it a potential drug target in cancer immunotherapy. The *c-CBL TR-FRET Assay Kit* is designed to measure c-CBL auto-ubiquitination activity in a homogeneous 384 reaction format. It utilizes Biotin=labeled Ubiquitin and a terbium-labeled anti-c-CBL antibody to complete the TR-FRET pairing. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80301	UBE1 (E1)	25 µg	-80°C	***Avoid freeze/ thaw cycles!***
80314	UBCH5b (E2)	200 µg	-80°C	
100370	Human c-CBL (E3), GST-tag	10 µg	-80°C	
	Biotin-Ubiquitin	400 µl	-80°C	
	ATP (4 mM)	1 ml	-80°C	
	CBL assay buffer	2 x 10 ml	-80°C	
	Tb-labeled donor	10 µl	-20°C	
	Dye-labeled acceptor	10 µl	-20°C	
	White, nonbinding Corning, low volume, microtiter plate	1	Room temp.	
	Plate sealer	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

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STABILITY: At least 6 months from date of receipt when stored as directed.

REFERENCE: 1. Lyle, C.L., *et al.*, *Cell*. 2019; **8(5):** E498.

ASSAY PROTOCOL:
All samples and controls should be tested in triplicates.

1) Thaw UBE1, UBCH5b, c-CBL, Biotin-Ubiquitin, c-CBL assay buffer, and ATP on ice. Aliquot each protein, assay buffer, and ATP into single-use aliquots and stored at -80°C immediately. *Note: UBE1, UBCH5b, c-CBL, Biotin-Ub, assay buffer, and ATP are sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

2) Carefully calculate the amount of proteins needed. Prepare appropriate amounts of diluted proteins as needed:

   Dilute the UBE1 in c-CBL assay buffer at 60 ng/µl.
   Dilute the UBCH5b in c-CBL assay buffer at 360 ng/µl.
   Dilute the C-CBL in c-CBL assay buffer at 2.3 ng/µl.

   Keep the diluted reagents on ice until use.

	Blank	Substrate Control	Positive Control	Test Inhibitor
Biotin-Ub	1 µl	1 µl	1 µl	1 µl
UBE1	1 µl	1 µl	1 µl	1 µl
UBCH5b	1 µl	1 µl	1 µl	1 µl
c-CBL	–	2.5 µl	2.5 µl	2.5 µl
Test Inhibitor/Activator	–	–	–	2 µl
Inhibitor buffer (no inhibitor)	2 µl	2 µl	2 µl	–
c-CBL assay buffer	2.5 µl	2.5 µl	–	–
ATP (4 mM)	2.5 µl	–	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

3) Prepare the master mixture using diluted reagents: N wells × (1 µl Biotin-Ub + 1 µl UBE1 + 1 µl UBCH5b + 2.5 µl c-CBL). Add 5.5 µl of master mixture to each well.

4) designated for the “Substrate Control”, “Positive Control”, “Test Inhibitor”. For the “blank”, add 1 µl Biotin-Ub+ 1 µl UBE1 + 1 µl UBCH5b + 2.5 µl assay buffer.

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5) Add 2 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 2 µl of the same solution without inhibitor (inhibitor buffer).

6) Initiate the reaction by adding 2.5 µl of ATP to the wells labeled “Positive Control”, “Test Inhibitor”, and “Blank”. Add 2.5 µl of assay buffer to the well designated “Substrate Control”. Incubate overnight at room temperature with slow shaking. Cover the plate with a plate sealer.

7) Dilute Tb-labeled donor (1:400) and Dye-labeled acceptor (1:400) together in one solution, using CBL Assay Buffer. Add 10 µl diluted donor/acceptor mixture into each well. Incubate at room temperature for three hours.

8) Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

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CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

Example of Assay Results:

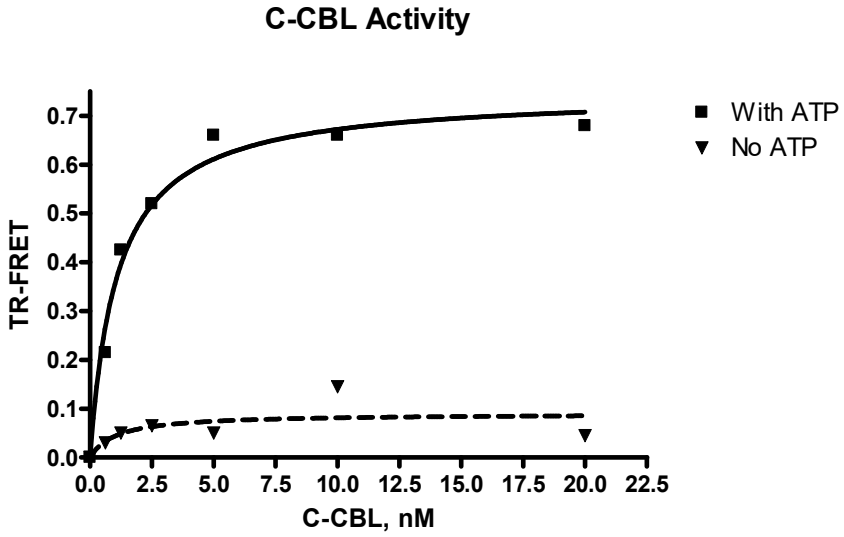

Figure 1: Titration of c-CBL activity using the *c-CBL TR-FRET Assay Kit*, BPS Bioscience #79786. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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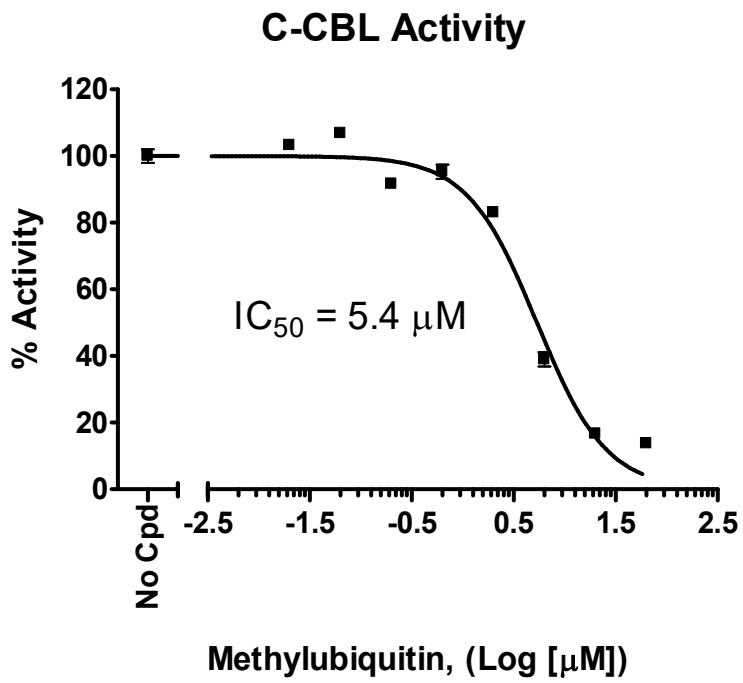

Figure 2: Inhibition of c-CBL Assay FRET signal by Methylated Ubiquitin, measured using the *c-CBL TR-FRET Assay Kit*, BPS Bioscience #79786. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	Catalog #	Size
c-CBL, GST-Tag	#100370	100 µg
CBL-C, FLAG-Tag	#100332	100 µg
CBL-B, GST-Tag (Human)	#80415	100 µg
CBL-B, His-Avi-Tag	#80414	100 µg
CBL-B, Biotin-labeled (Human)	#80412	50 µg
CBL-B (Y363F), Biotin-labeled (Human)	#80413	50 µg
UBE1 (UBA1), FLAG-tag	#80301	100 µg
UBCH5b	#80314	100 µg
Ubiquitin, His-Avi-Tag, Biotin Labeled	#11236	50 µg
CBL-B TR-FRET Assay Kit	#79575	96 rxns.