



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](https://www.linkedin.com/company/szaboscandic) 



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

Data Sheet
CBL-B TR-FRET Assay Kit
Catalog # 79575
Size: 384 reactions

DESCRIPTION:

Human Casitas B-lineage lymphoma proto-oncogene b (CBL-B) is an E3 ubiquitin-protein ligase that functions as a negative regulator of T-cell activation. It is a promising drug target in cancer immunotherapy. The *CBL-B TR-FRET Assay Kit* is designed to measure CBL-B auto-ubiquitination activity in a homogeneous 384 reaction format. It utilizes biotin-labeled Ubiquitin and a terbium-labeled antibody recognizing the GST-tagged CBL-B protein 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)	5 µg	-80°C	Avoid freeze/thaw cycles!
80314	UBCH5b (E2)	200 µg	-80°C	
80415	Human CBL-B (E3), GST-tag	10 µg	-80°C	
	Biotin-Ubiquitin	400 µl	-80°C	
	ATP (400 µM)	150 µl	-80°C	
	CBL assay buffer 2	2 x 10 ml	-80°C	
	Tb-labeled donor	10 µl	-20°C	
	Dye-labeled acceptor	10 µl	-20°C	
	Methyl-ubiquitin (1 mM in 50 mM Tris, pH 7.4)	20 µl	-80 °C	
	White Corning microtiter plate	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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



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

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

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

REFERENCE: 1. Jeon, M.S., *et al.*, *Immunity*. 2004; **21(2)**: 167-177.
2. Chiang, Y.J. *et al.*, *Nature*. 2000; **403**: 216-220.

ASSAY PROTOCOL:

All samples and controls should be tested in triplicates.

- 1) Thaw **UBE1**, **UBCH5b**, **CBL**, **Biotin-Ubiquitin**, **CBL assay buffer**, and **ATP** on ice. Aliquot each protein, **CBL assay buffer**, and **ATP** into single-use aliquots and stored at -80°C immediately. *Note: **UBE1**, **UBCH5b**, **CBL-B**, **Biotin-Ub**, **CBL-B assay buffer 2**, 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; dilute only the amount required for the assay. Do not store diluted proteins

Dilute the **UBE1** in **CBL assay buffer 2** at 12.5 ng/ μl
Dilute the **UBCH5b** in **CBL assay buffer 2** at 450 ng/ μl
Dilute the **CBL-B** in **CBL assay buffer 2** at 3 ng/ μl
Keep the diluted reagents on ice until use.
- 3) Prepare the master mixture using diluted reagents: N wells \times (1 μl **Biotin-Ub** + 1 μl diluted **UBE1** + 1 μl diluted **UBCH5b** + 2.5 μl diluted **CBL-B**). Add 5.5 μl of master mixture to each well designated for the "Substrate Control," "Positive Control," "Test Inhibitor." For the "blank," add 1 μl **Biotin-Ub** + 1 μl diluted **UBE1** + 1 μl diluted **UBCH5b** + 2.5 μl **CBL assay buffer 2**.
- 4) Add 2 μl of inhibitor solution of each well designated "Test Inhibitor." For the "Positive Control," "Substrate Control," and "Blank," add 2 μl of 1% DMSO in water (inhibitor buffer).
- 5) Dilute the **ATP stock** (400 μM) 10-fold using **CBL assay buffer 2** to 40 μM ; dilute only the amount required for the assay. Initiate the reaction by adding 2.5 μl of diluted **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 the reaction at 30°C for four hours. Cover the plate with a plate sealer if necessary.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



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

	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
CBL-B	-	2.5 µl	2.5 µl	2.5 µl
Test Inhibitor/Activator	-	-	-	2 µl
1% DMSO in water (Inhibitor buffer)	2 µl	2 µl	2 µl	-
CBL assay buffer 2	2.5 µl	2.5 µl	-	-
ATP (40 µM)	2.5 µl	-	2.5 µl	2.5 µl
Total	10 µl	10 µl	10 µl	10 µl

- 6) Dilute **Tb-labeled donor** (1:400) and **Dye-labeled acceptor** (1:400) in one step using CBL Assay Buffer 2. Prepare only the amount required for the assay. Add 10 µl of diluted donor/acceptor mixture into each well. Incubate at room temperature for one hour.
- 7) Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET. Blank value is subtracted from all other values.

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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



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

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.

$$\% \text{ Activity} = \frac{\text{FRET}_s - \text{FRET}_{\text{neg}}}{\text{FRET}_p - \text{FRET}_{\text{neg}}} \times 100\%$$

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

Example of Assay Results:

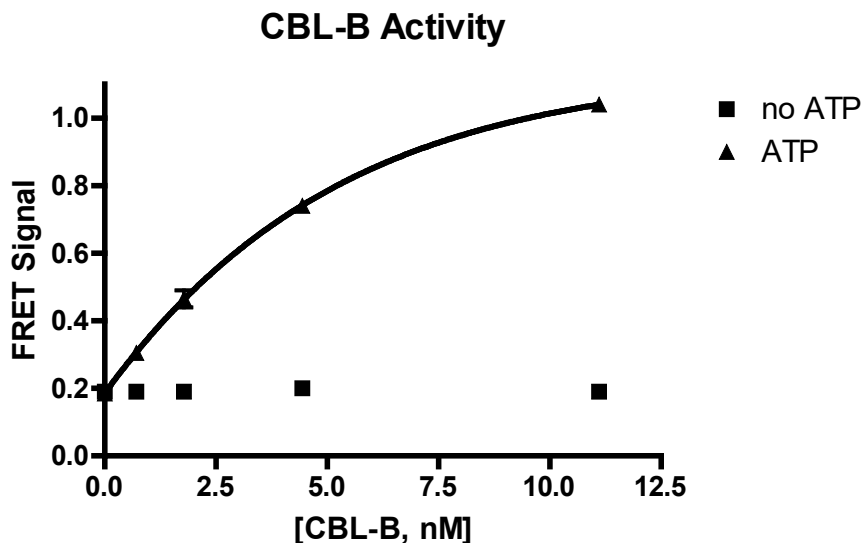


Figure 1: Titration of CBL-B activity using the *CBL-B TR-FRET Assay Kit*, BPS Bioscience #79575. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com

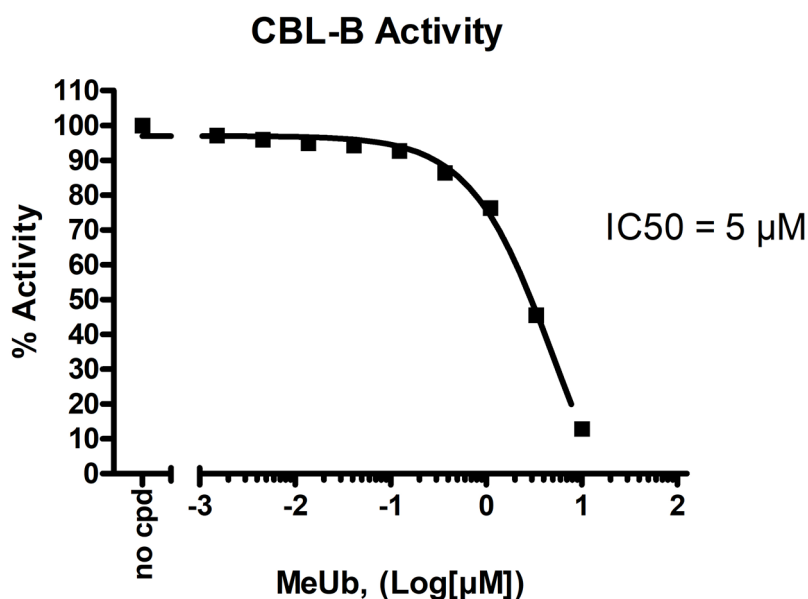


Figure 2: Inhibition of CBL-B Assay FRET signal by Methylated Ubiquitin, measured using the *CBL-B TR-FRET Assay Kit*, BPS Bioscience #79575. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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
UbcH5a, His-Tag (Human)	#80315	100 μg
UbcH5c, His-Tag (Human)	#80313	100 μg
UBA6 (UBE1L2), FLAG-tag	#80303	100 μg

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com