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

Data Sheet

UBE1 Inhibitor Screening Assay Kit

Catalog #79957
96 Reactions

DESCRIPTION: UBE1 dysregulation has been linked to muscular atrophy, tumors, and cancer. The *UBE1 Inhibitor Screening Assay Kit* is designed to measure UBE1 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *UBE1 Inhibitor Screening Assay Kit* comes in a convenient 96-well format, with enough purified recombinant UBE1 enzyme, Ubiquitin, ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
80301	UBE1, FLAG-tag	150 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	50 µl	-20°C	
	Ubiquitin (500 µM)	100 µl	-20°C	
79696	96-well plate, white	1	RT	

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 0.5 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

REFERENCES:
1. Ramser, J., *et al.* 2008. "Rare missense and synonymous variants in UBE1 are associated with X-linked infantile spinal muscular atrophy." *Amer. J.Human Genetics* **82(1):** 188-193.
2. Leidecker, O., *et al.* 2012. "The ubiquitin E1 enzyme Ube1 mediates NEDD8 activation under diverse stress conditions." *Cell Cycle* **11(6):** 1142-1150.

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

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Ubiquitin**.
(Optional: If desired, add 30 µl of 0.5 M DTT to 1.5 ml **5x Kinase assay buffer**).

2) Prepare the master mixture (25 µl per well): N wells x (10 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 1 µl **Ubiquitin** + 13.5 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	10 µl	10 µl	10 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
Ubiquitin	1 µl	1 µl	1 µl
Water	13.5 µl	13.5 µl	13.5 µl
Test Inhibitor	–	5 µl	–
Inhibitor buffer	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
UBE1, FLAG-tag (75 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Add 5 µl of Inhibitor solution to- each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer, usually 10% DMSO in water).

Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.

4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

6) Thaw **UBE1, FLAG-tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **UBE1, FLAG-tag** required for the assay and dilute enzyme to 10 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note:*

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

UBE1, FLAG-tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

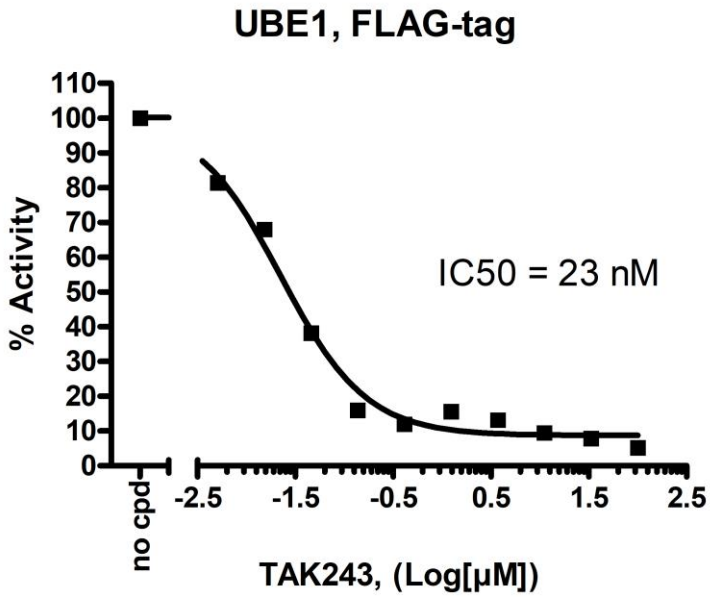
7) Initiate reaction by adding 20 µl of diluted **UBE1, FLAG-tag** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45-minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

10) Measure luminescence using the microplate reader. Value of “Blank” reading should be subtracted from all other measurements.

Example of Assay Results:



Inhibition of UBE1, FLAG-tag by TAK243, measured using the UBE1Inhibitor Screening Assay Kit (BPS Bioscience #79957). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@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

RELATED PRODUCTS:

Product Name	Catalog #	Size
UBE1, FLAG-tag	80301	100 µg
Ubiquitin, His-Tag	79293	2 mg
5x Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
UBA6 (UBE1L2), FLAG-tag	80303	100 µg
UBE2A, His-Tag	79368	20 µg
UBE2C, His-Tag	79369	20 µg
UBE3A, His-FLAG-tags	80302	20 µg
CBL-B TR-FRET Assay Kit	79575	384 rxns.