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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.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **5x Kinase assay buffer**, **ATP (500 μ M)**, and **Ubiquitin**.
(Optional: If desired, add 30 μ l of 0.5 M DTT to 1.5 ml **5x Kinase assay buffer**).
- 2) Prepare the master mixture (25 μ l per well): N wells x (10 μ l **5x Kinase assay buffer** + 0.5 μ l **ATP (500 μ M)** + 1 μ l **Ubiquitin** + 13.5 μ l distilled water). Add 25 μ l to every well.

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

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

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

- 4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μ l of **5x Kinase assay buffer** with 2400 μ l water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank," add 20 μ l 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/ μ l with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note:* OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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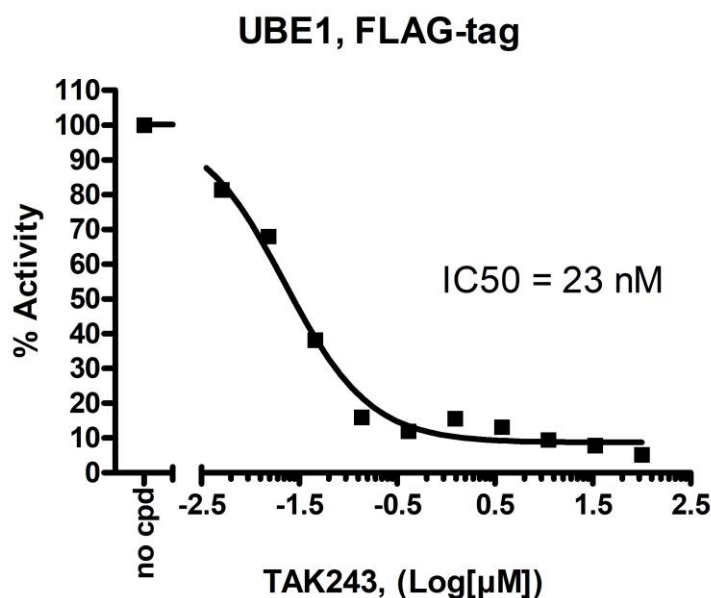
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UBE1, FLAG-tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

- 7) Initiate reaction by adding 20 μ l 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 μ l 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*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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.

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