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

Data Sheet

Ataxin-3 Fluorescent Assay Kit

Catalog #78044
Size: 96 reactions

BACKGROUND: Human Ataxin-3 (ATX3 or ATXN3), also known as Josephin (JOS) or Machado-Joseph disease protein 1 (MJD1), is implicated in Machado-Joseph disease, a neurological disease that progresses cerebellar ataxia. It is also clinically relevant in gastric, lung, and testicular cancers.

DESCRIPTION: The *Ataxin-3 Fluorescent Assay Kit* is designed to measure Ataxin-3 activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit comes in a convenient 96-well format, with purified Ataxin-3 protein, Ubiquitin-AMC, and Ataxin-3 assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80399	Ataxin-3	500 µg	-80°C	***Avoid freeze/ thaw cycles!***
81150	Ub-AMC (10 µM)	200 µl	-80°C	
78045	Ataxin-3 Assay Buffer	5 ml	-20°C	
79685	Black, low binding black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading λexc/λem=360 nm/460 nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCES:

1. Shi, Z., *et al.* 2018. Ataxin-3 promotes testicular cancer cell proliferation by inhibiting anti-oncogene PTEN. *Biochem Biophys Res Commun.* **503(1):**391-396. doi:10.1016/j.bbrc.2018.06.047
2. Koch, P., *et al.* 2011. Excitation-induced ataxin-3 aggregation in neurons from patients with Machado–Joseph disease. *Nature* **480(7378):** 543-546.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Dilute **Ub-AMC (10 µM)** in water to make an 800 nM solution. Dilute only enough as is required for the assay. Store remaining 10 µM substrate in aliquots at -80°C.

2) Add 25 µl of **Ub-AMC (800 nM)** to each well (Final concentration of the Ub-AMC in a 50 µl reaction is 400 nM).

Component	Positive Control	Test Sample	Blank
Ub-AMC (800 nM)	25 µl	25 µl	25 µl
Test Inhibitor	–	5 µl	–
Inhibitor buffer (usually 10% DMSO in assay buffer)	5 µl	–	5 µl
Ataxin-3 Assay Buffer	–	–	20 µl
Ataxin-3 (250 ng/µl)	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

3) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in assay buffer (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). To determine an IC50 or to test lower concentrations of the compound, prepare a series of further dilutions in assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in assay buffer.

4) Add 5 µl inhibitor solution to each well designated “Test Sample.” Add 5 µl of inhibitor buffer (usually 10% DMSO in assay buffer) to “Blank” and “Positive Control” wells.

5) Add 20 µl **Ataxin-3 Assay Buffer** to the “Blank” wells.

6) Thaw **Ataxin-3** on ice. Upon first thaw, briefly spin tube containing protein to recover the full content of the tube. Aliquot **Ataxin-3** into single use aliquots.

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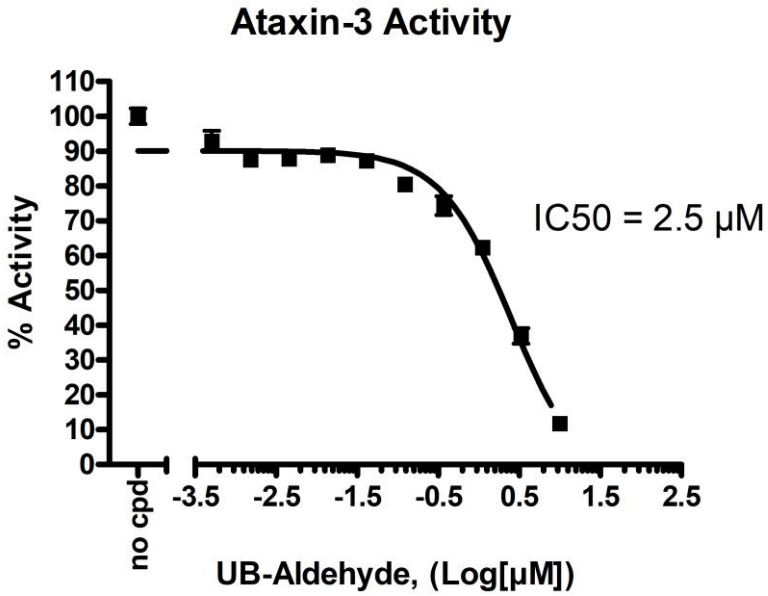
Store remaining undiluted protein in aliquots at -80°C. Note: **Ataxin-3** is sensitive to freeze/thaw cycles. Do not re-use diluted protein.

7) Dilute **Ataxin-3** in **Ataxin-3 Assay Buffer** at 250 ng/µl (5 µg per reaction).

8) Add 20 µl diluted **Ataxin-3** solution to wells designated as "Positive Control" and "Test Sample."

9) Incubate at 37°C for 20 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at wavelength 360 nm and detection of emission at wavelength 460 nm. The fluorescence intensity can also be measured kinetically. "Blank" value is subtracted from all other values.

EXAMPLE OF ASSAY RESULTS:



Ataxin-3 inhibition by Ub-Aldehyde (Boston Biochem, #U-550-50), measured using the Ataxin-3 Fluorescent Assay Kit, BPS Bioscience, #78044. Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at *info@bpsbioscience.com*.

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RELATED PRODUCTS

Product	Cat. #	Size
Ataxin3 (MJD1, SCA3), His-tag	80399	250 µg
Ubiquitin AMC	81150	50 µg
Ubiquitin, His-Tag	79293	2 mg
Ubiquitin Rhodamine	81151	50 µg
Ubiquitin, His-Avi-Tag, Biotin Labeled	11236	50 µg
Ataxin3-Like, His-tag	81082	25 µg
JosD1, His-tag	81085	25 µg
JosD2, His-tag	81086	25 µg