

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	<i>Avoid freeze/ thaw cycles!</i>
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 $\lambda_{exc}/\lambda_{em}=360\text{ nm}/460\text{ nm}$

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 μ M)** in water to make an 800 nM solution. Dilute only enough as is required for the assay. Store remaining 10 μ M substrate in aliquots at -80°C.
- 2) Add 25 μ l of **Ub-AMC (800 nM)** to each well (Final concentration of the Ub-AMC in a 50 μ l reaction is 400 nM).

Component	Positive Control	Test Sample	Blank
Ub-AMC (800 nM)	25 μ l	25 μ l	25 μ l
Test Inhibitor	–	5 μ l	–
Inhibitor buffer (usually 10% DMSO in assay buffer)	5 μ l	–	5 μ l
Ataxin-3 Assay Buffer	–	–	20 μ l
Ataxin-3 (250 ng/ μ l)	20 μ l	20 μ l	–
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 μ l inhibitor solution to each well designated “Test Sample.” Add 5 μ l of inhibitor buffer (usually 10% DMSO in assay buffer) to “Blank” and “Positive Control” wells.
- 5) Add 20 μ l **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.

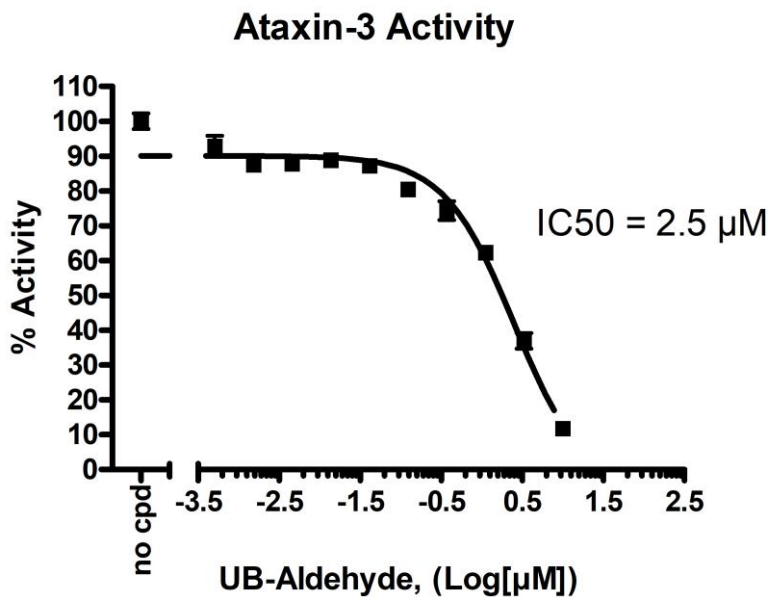
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Store remaining undiluted protein in aliquots at -80°C . Note: **Ataxin-3** is sensitive to freeze/thaw cycles. Do not re-use diluted protein.

- 7) Dilute **Ataxin-3** in **Ataxin-3 Assay Buffer** at $250\text{ ng}/\mu\text{l}$ ($5\text{ }\mu\text{g}$ per reaction).
- 8) Add $20\text{ }\mu\text{l}$ 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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
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

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