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Data Sheet
A20 Fluorogenic Assay Kit
Catalog #78024
Size: 96 reactions

BACKGROUND: A20 or TNFAIP3 is a protein that is integral to controlling NF-kB activation. It was first identified in human umbilical vein endothelial cells, but has now been induced in other cell types. A20 has been implicated in rheumatoid arthritis and other autoinflammatory and autoimmune diseases. It also plays a role in cytokine-induced signaling pathways.

DESCRIPTION: The *A20 Fluorogenic Assay Kit* is designed to measure A20 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 A20 protein, Ubiquitin-AMC, and A20 assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80394	A20, FLAG-tag	20 µg	-80°C	<i>Avoid freeze/ thaw cycles!</i>
81150	Ub-AMC (10 µM)	500 µl	-80°C	
78025	A20 Assay Buffer	10 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.

REFERENCE(S):

1. Kato, M., *et al.* 2009. "Frequent inactivation of A20 in B-cell lymphomas." *Nature* **459(7247)**: 712-716.
2. Boone, D.L., *et al.* 2004. "The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses." *Nature Immunology* **5(10)**: 1052-1060.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Prepare the master mixture: N wells × (65 µl **A20 Assay Buffer** + 5 µl **Ub-AMC (10 µM)**).
- 2) Add 70 µl of the master mixture to each well (Final concentration of the **Ub-AMC** in a 100 µl reaction is 500 nM).

Component	Positive Control	Test Sample	Blank
Ub-AMC (10 µM)	5 µl	5 µl	5 µl
A20 Assay Buffer	65 µl	65 µl	85 µl
Test Inhibitor	–	10 µl	–
Inhibitor Buffer (10% DMSO in assay buffer)	10 µl	–	10 µl
A20, FLAG-tag (9 ng/µl)	20 µl	20 µl	–
Total	100 µl	100 µl	100 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 A20 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 10 µl inhibitor solution to each well designated “Test Sample.” Add 10 µl of inhibitor buffer (10% DMSO in A20 assay buffer) to “Blank” and “Positive Control” wells.
- 5) Thaw **A20, FLAG-tag** on ice. Upon first thaw, briefly spin tube containing protein to recover the full content of the tube. Aliquot **A20, FLAG-tag** into single use aliquots. Store remaining undiluted protein in aliquots at -80°C. Note: **A20, FLAG-tag** is sensitive to freeze/thaw cycles. Do not re-use diluted protein.
- 6) Dilute **A20, FLAG-tag** in **A20 Assay Buffer** at 9 ng/µl (180 ng per reaction).

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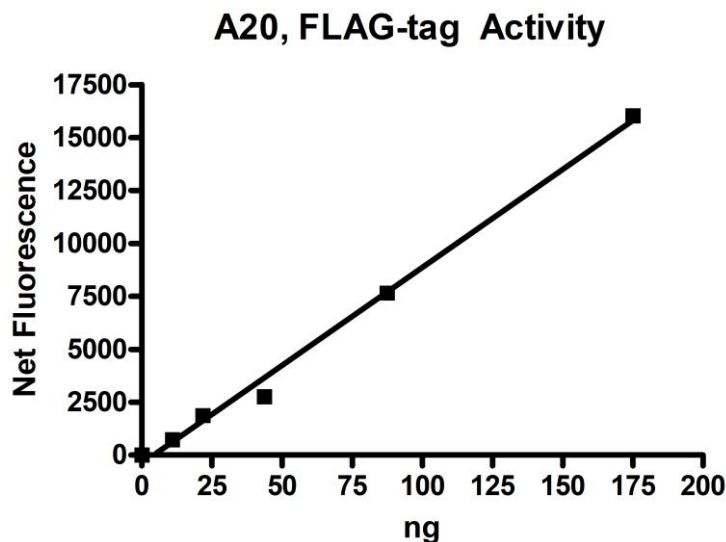
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- 7) Add 20 μ l diluted **A20, FLAG-tag** solution to wells designated as "Positive Control" and "Test Sample." Add 20 μ l **A20 Assay Buffer** to the "Blank" wells.
- 8) Incubate at 37°C for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength 360 nm and detection of emission at a wavelength 460 nm. The fluorescence intensity can also be measured kinetically. "Blank" value is subtracted from all other values.

EXAMPLE OF ASSAY RESULTS:



A20 activity, measured using the A20 Fluorogenic Assay Kit, BPS Bioscience #78024. Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
A20, FLAG-tag (Sf9-derived)	80394	50 μ g
A20, His tag, FLAG-tag (Sf9-derived)	80408	50 μ g
A20, His-tag (E. coli-derived)	80393	100 μ g
Ubiquitin AMC	81150	50 μ g
Tumor Necrosis Factor-alpha (Human)	90244-A	10 μ g
Tumor Necrosis Factor-alpha (Human)	90244-B	50 μ g
NF- κ B reporter (Luc) - HEK293 Cell line	60650	2 vials
NF- κ B Reporter Kit (NF- κ B Signaling Pathway)	60614	500 rxns.

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