MA-0156

Neutrophil Elastase Activity Assay

(100 wells, Fluorometric, Ex/Em = 380/500 nm, Store at -20°C)

Background:

Neutrophil elastase is a key component of the immune response. The primary function of neutrophil elastase is to degrade bacterial components at the site of infection, contributing to the breakdown of the bacterial cell wall. In inflammatory disease conditions, neutrophil elastase becomes dysregulated, being released inappropriately by the neutrophils and degrading ECM proteins which are essential for tissue integrity and function. This is seen in conditions such as COPD, ARDS and autoimmune disorders like rheumatoid arthritis. The ability to selectively target neutrophil elastase with inhibitors in several disease states could mitigate excessive tissue destruction while preserving its activity in infections. The ability to measure neutrophil elastase activity is essential in this work. AkrivisBio's Neutrophil Elastase Activity Assay is a simple, sensitive means of assessing elastase activity in a variety of biological sample types with a sensitivity below 50 µU in a sample well.

Assay Principle:

Neutrophil elastase cleaves a highly specific fluorogenic substrate which releases AFC providing intense fluorescence.

Assay Components:

Assay Buffer	15 ml	WM	MA-0156A
NE Dilution Buffer	1 ml	Clear	MA-0156B
MeOSucc-AAPV-AFC	0.2 ml	Red	MA-0156C
AFC Standard	0.5 ml	Yellow	MA-0156D
Neutrophil Elastase	lyoph	Green	MA-0156E

Storage and Handling Considerations:

Store unopened kit at -20°C. Centrifuge all small vials briefly before opening.
Assay Buffer, Dilution Buffer: Ready to use as supplied. Bring to room temperature before use. Store at 4°C
MeOSucc-AAPV-AFC: Light sensitive. Bring to room temperature before using to melt DMSO. Store at -20°C
AFC Standard: 10 µM AFC in DMSO. Ready to use as supplied. Store at 4°C
Neutrophil Elastase Positive Control: Reconstitute with 10 µl of Dilution Buffer to prepare a 100 µg/ml stock solution. Aliquot immediately and store at -80°C. Avoid repeated freeze/thaw.

Assay Protocol:

1. Prewarm plate reader to 37°C.

2. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the AFC Standard to a series of wells in a 96-well plate. Adjust all wells to 100 µl with Assay Buffer giving 0 – 50 – 100 – 150 – 200 – 250 pmoles of AFC.

3. Samples: Liquid samples (purified enzyme, blood, plasma) may be added directly to wells in a 96-well plate. Transfer up to 50 µl of sample per well and adjust all wells to 50 µl with Assay Buffer.

4. Neutrophil Elastase Positive Controls: Dilute 1-2 µl of the reconstituted enzyme 100X. Transfer 1 – 5 µl of diluted enzyme to wells in a 96-well plate. Adjust the volume to 50 µl with Assay Buffer.

5. Place the plate in the plate reader while the Reaction Mix is being prepared.

Note: Unlike other kit suppliers, we don't assume 100% integrity of our positive control enzyme. Rather we create a standard curve of known amounts of AFC and determine the actual activity from the observed enzymatic reaction rate.

6. Initiate Reaction: Each sample and positive control well will require 50 µl of Reaction Mix. Prepare sufficient material for the number of samples and positive control wells to be analyzed containing:

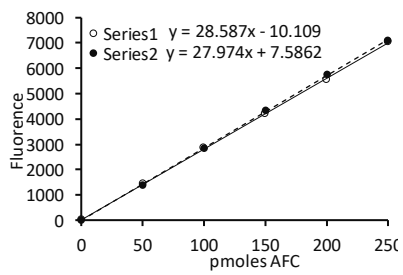
	Reaction Mix
Assay Buffer	48 µl
MeOSucc-AAPV-AFC	2 µl

Add 50 µl of Reaction Mix into all Sample and Positive control wells.

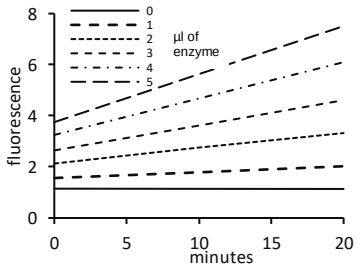
7. Measurement: Monitor reaction rate in kinetic mode for 30 min. at 37°C using excitation 380 nm, emission 500 nm. Allow the reactions to proceed long enough to observe a clear linear reaction rate.

8. Typical Results:

pmole Standard	Standard Raw Values RFU		Background Corrected Values RFU	
0	33.939	35.866	0	0
50	1451.633	1416.273	1417.694	1380.407
100	2859.608	2878.797	2825.669	2842.931
150	4227.378	4349.118	4193.439	4313.252
200	5586.920	5794.355	5552.981	5758.489
250	7070.060	7120.420	7036.121	7084.554



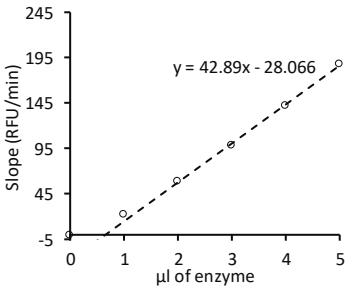
Standard Curve



Reaction Rates for different Amounts of Positive Control

µl enzyme	slope
0	-0.599
1	23.051
2	59.629
3	98.301
4	142.360
5	187.910

Slopes of different amounts



Slope vs. µl of Positive Control

In this example, the standard gave 28 RFU/pmol of AFC. The enzyme rate is 43 RFU/minute or ~ 1.5 pmol/min/µl of enzyme.

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9. Calculations: Subtract 0 Standard reading from all readings. Plot the AFC Standard Curve. Determine the slope of the AFC Standard Curve. This defines the sensitivity of the assay system. Determine the slope of the Positive Control and of the test samples. To convert from RFU/ minute to pmoles/minute (µU), divide the slope of the Positive Control by the slope of the Standard Curve. To convert from values in the well to amounts per amount of sample:
Divide the amount of enzyme in the well by the volume in µl added to the well = µU per µl of sample.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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