MA-0128

Trypsin Activity Assay

(100 wells, Colorimetric, OD 405 nm, Store at 4°C)

Introduction:

Trypsin, a serine protease produced in the pancreas, in an inactive proenzyme form. Secreted into the small intestine, the proenzyme is activated by enterokinase. Trypsin favors cleavage in peptides on the carboxyl side of basic amino acids (lysine, arginine). Trypsin is used in several biotechnology applications such as cell and tissue culture, peptide sequencing and cell dissociation. AkrivisBio's Trypsin Assay is a kinetic assay which utilizes a trypsin substrate (GPK-pNA). When cleaved by trypsin,*p*-nitroaniline is released which is intensely colored with an absorbance maximum 405 nm allowing for detection of trypsin activity as low as 0.05 – 0.1 mU per sample.

Assay Principle:

1 -Trypsin cleaves GPK-pNA, releasing free pNA into the solution
2 – pNA content, with an extinction coefficient of 6.993 mM^{-1} $cm^{-1,}$ is determined by absorbance

Assay Components:

Assay Buffer	25 ml	WM	MA-0128-A
GPK-pNA Solution	200 µl	Red	MA-0128_B
Trypsin Positive Control	lyophilized	Blue	MA-1028-C
p-NA Standard (0.8 mM)	400 µl	Yellow	MA-1028-D
Trypsin Inhibitor	100 µl	Purple	MA-0128-E
Chymotrypsin Inhibitor	100 µl	White	MA-0128-F

Storage and Handling:

Store at 4°C. Centrifuge all vials for a few seconds before opening. Warm all components to room temperature before using.
GPK-pNA, *p*-NA Standard, Trypsin Inhibitor and Chymotrypsin Inhibitor: Ready to use as supplied.
Trypsin Positive Control: Dissolve with 100 µl Assay Buffer. If the assay is to be used repeatedly of a period of time, aliquot the enzyme into convenient portions and store at -20°C.

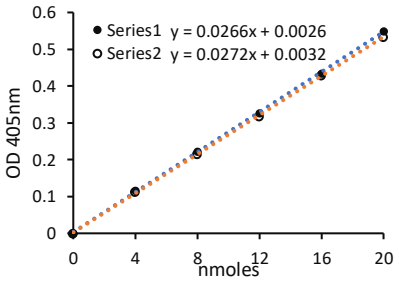
Assay Protocol:

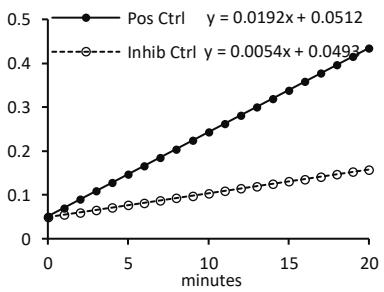
1. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl *p*-NA standard to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer giving 0, 4, 8, 12, 16, and 20 nmol/well of the *p*-NA standard.
2. Positive Control: Transfer 5 µl to 95 µl DI water. Add 5 µl positive control to paired wells, adjust volume to 50 µl/well with Assay Buffer. Add 1 µl of trypsin inhibitor to one of the wells and preincubate for 5 minutes to give a trypsin inhibitor comparison control.
3. Sample Preparation and Use: Extract tissue (10 mg) or cells (10^6) with 100 µl of Assay Buffer, centrifuge at 16,000 X g for 5 min. Transfer 20 - 50 µl of sample to a 96 well plate and adjust all well volume to 50 µl with Assay Buffer. For serum, add 50 µl to sample well. It is important that unknown samples give readings within the range of the standard curve. If any sample is outside this range, dilute appropriately and rerun. Correct for non-trypsin activity, by treating a paired sample with 1 µl of chymotrypsin inhibitor (TPCK) solution and preincubate for 10 minutes before starting the reaction by the addition of GPK-pNA.
4. Initiate Reaction: Each reaction requires 50 µl of reaction mix. Prepare sufficient reaction mix for the number of wells to be run:

Reaction Mix:	
Assay Buffer	48 µl
Trypsin Substrate	2 µl

Mix and add 50 µl to each well to be run (*p*-NA standards, positive control, test samples, test sample trypsin inhibitor control).
5. Measurement: Monitor all wells in kinetic mode for up to 2 hours at 405 nm at room temperature. This can be extended if activity is low.
Note: This is a kinetic assay in which the reaction rate as determined by change in absorbance per unit time is proportional to the amount of active trypsin present in the well. The data should be examined to determine the portion of the measurement where the change is **linear with time.**
Once the linear portion has been determined,
6. Typical Results:

	Standard Raw Values		Background Corrected Values	
nmol Standard	OD			
0	0.1909	0.1839	0	0
4	0.2843	0.2807	0.0934	0.0968
8	0.3861	0.3863	0.1952	0.2024
12	0.4881	0.4912	0.2972	0.3073
16	0.5997	0.5995	0.4088	0.4156
20	0.7047	0.7134	0.5138	0.5295





7. Calculation: Subtract the 0 Standard from all other standards. Plot the *p*-NA standard Curve and determine the slope (OD/nmol). For all other wells, determine the linear slope of each reaction (OD/minute). Divide each reaction rate by the slope of the standard curve for each positive control, test sample and inhibitor control (OD/minute)/(OD/nmol) = (nmol/minute or mU) in the well. Apply corrections to convert this value to trypsin activity per sample as follows:
A. mU in well / µl sample added to well = mU Trypsin activity/ µl sample
B. mU Trypsin activity/ µl sample X volume of sample in µl = total mU per sample.
C. total mU per sample / mg tissue (or # of cells or µl serum) = mU per mg (or # of cells or µl serum)

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