Product Information

Trypsin Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2176-100 100 assays	Cap Color	Part Number
Trypsin Assay Buffer	25 ml	WM	K2176-C-1
Trypsin Substrate (in DMSO)	200 µl	Red	K2176-C-2
Positive Control (lyophilized)	1 vial	Blue	K2176-C-3
p-NA Standard (2 mM)	400 µl	Yellow	K2176-C-4
Trypsin Inhibitor (TLCK, 20 mM)	100 µl	Purple	K2176-C-5
Chymotrypsin Inhibitor (TPCK,10 mM)	100 µl	Clear	K2176-C-6

II. Introduction:

Trypsin is a serine protease that hydrolyses proteins in the digestive system of various vertebrates. Pancreas produces trypsin as inactive proenzyme trypsinogen. Active trypsin cleaves peptide chains predominantly at the carboxyl side of the amino acids (lysine or arginine). In the Trypsin Activity Colorimetric Assay, trypsin cleaves a substrate to produce p-nitroaniline (p-NA) that is detectable atλ=405 nm. Trypsin activity can be measured as the color intensity is proportional to p-NA content. The kit detection limit is 10-100 mU (p-NA unit) trypsin in different samples.

III. Reagent Preparation and Storage Conditions:

Trypsin Substrate, p-NA Standard, Trypsin Inhibitor and Chymotrypsin Inhibitor are in DMSO solution, need to be warmed up to room temperature to become solution before use.

Positive Control: Dissolve with 100 µl Assay Buffer. Pipette up and down to completely dissolve, aliquot and store at -20°C. Use within two months. Prevent from freeze/thaw cycle.

IV. Trypsin Activity Assay Protocol:

1. Standard Curve Preparations:

Add 0, 2, 4, 6, 8, 10 µl p-NA standard into a series of standards wells. Adjust volume to 50 µl/well with Trypsin Assay Buffer to generate 0, 4, 8, 12, 16, and 20 nmol/well of the p-NA standard.

2. Sample and Positive Control Preparations: Tissues or cells can be extracted with 4 volumes of the Trypsin Assay Buffer, centrifuge in micro-centrifuge at top speed for 10 min to get a clear extract. Prepare test samples at 50 µl/well with Assay Buffer in a 96-well plate. Serum can be directly added into sample wells, and the volume adjusted to 50 µl/well with Assay Buffer. We suggest using several doses of your sample to ensure the readings are within the linear range. Treat with 1 µl of 50X chymotrypsin inhibitor (TPCK) solution and incubate for 10 minutes at room temperature. For the positive control, add 5 µl positive control solution to wells, adjust volume to 50 µl/well with Assay Buffer. If desired, set a trypsin inhibitor sample group as a control by adding 1 µl of 50X trypsin inhibitor (TLCK) solution to trypsin inhibitor sample control and incubate for 5 min.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer 48 µl

Trypsin Substrate 2 µl

Mix well and add 50 µl of the reaction mix to each well containing the p-NA standards, positive controls, test samples or test samples trypsin inhibitor control, mix well, incubate at 25°C, protected from light.

4. Initially measure absorbance at 405 nm at time T1 (A1 and A1C for trypsin inhibitor control). After incubating the reaction for 1-2 hours (or incubate longer time if the trypsin activity is low) measure the absorbance at T2 (A2 and A2C). The color generated by cleavage of substrate is ΔA405nm = (A2 – A2C) – (A1 – A1C) or (A2 – A1), if no trypsin inhibitor control was run.

Note: It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1 and A2 in the reaction linear range.

5. Calculation: Subtract 0 Standard from all readings. Plot the p-NA standard Curve. Apply the ΔA405nm to the standard curve to get the nmol of p-NA (amount generated between T1 and T2 in the reaction wells).

Trypsin Activity = B/[(T2-T1) × V] × Sample Dilution Factor = nmol/min/ml = mU/ml

Where: B is the p-NA calculated from the Standard Curve (in nmol).

T1 and T2 are the times of the first and second readings (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition: One unit is defined as the amount of trypsin that cleaves the substrate, yielding 1.0 µmol of p-NA per minute at 25°C.

Note: 1 p-NA Unit = 0.615 TAME Unit = 35 BAEE Unit.

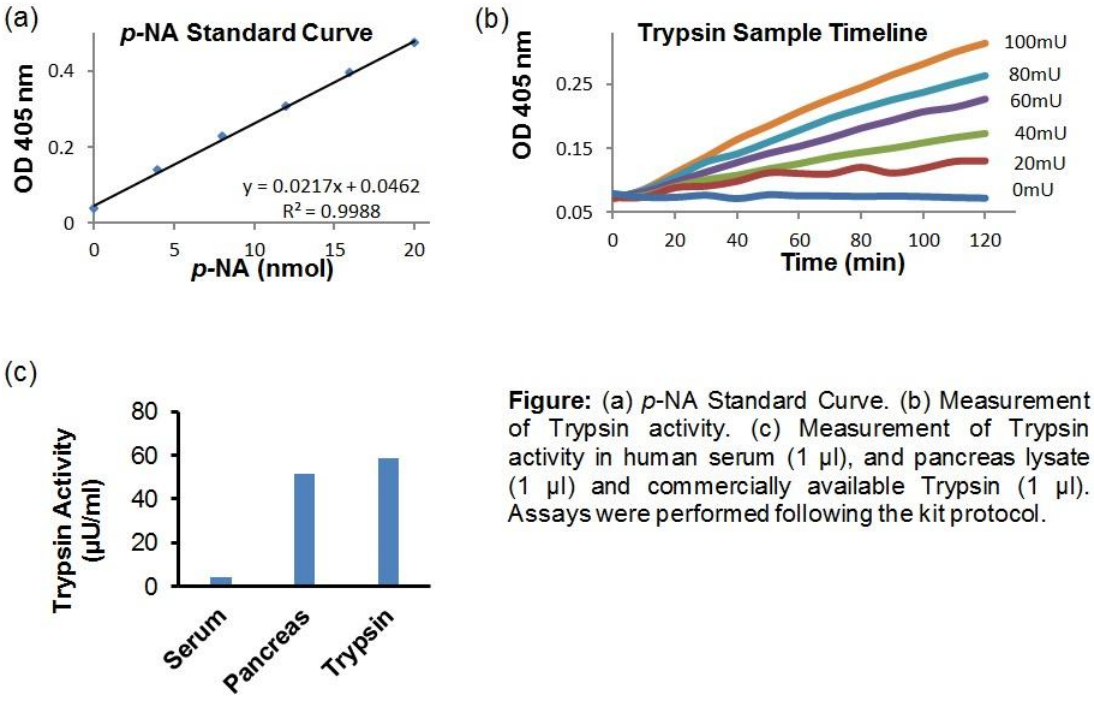

Figure: (a) *p*-NA Standard Curve. (b) Measurement of Trypsin activity. (c) Measurement of Trypsin activity in human serum (1 µl), and pancreas lysate (1 µl) and commercially available Trypsin (1 µl). Assays were performed following the kit protocol.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

	• Use of a different 96-well plate	of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

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