Trypsin Activity Colorimetric Assay Kit

(Catalog #BN00984; 100 assays; Store kit at –20˚C)

I. Introduction:

Trypsin (EC 3.4.21.4) is a serine protease found in the digestive system of many vertebrates, where it hydrolyses proteins. Trypsin is produced in the pancreas as the inactive proenzyme trypsinogen. Active trypsin predominantly cleaves peptide chains at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline. It is used for numerous biotechnological processes. In the assay, trypsin cleaves a substrate to generate *p*-nitroaniline (*p*-NA) which is detected at λ = 405 nm. Since the color intensity is proportional to *p*-NA content, trypsin activity can be accurately measured. The kit detects 10 - 100 mU (*p*-NA unit) trypsin in various samples.

II. Kit Contents:

Components	BN00984	Cap Code	Part Number
Trypsin Assay Buffer	25 ml	WM	BN00984-1
Trypsin Substrate (in DMSO)	200 µl	Red	BN00984-2
Positive Control (lyophilized)	1 vial	Blue	BN00984-3
p-NA Standard (2 mM)	400 µl	Yellow	BN00984-4
Trypsin Inhibitor (TLCK, 20 mM)	100 µl	Purple	BN00984-5
Chymotrypsin Inhibitor (TPCK,10 mM)	100 µl	White	BN00984-6

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:

48 µl Assay Buffer
2 µl Trypsin Substrate

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 T_1 (A_1 and A_{1C} 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 T_2 (A_2 and A_{2C}). The color generated by cleavage of substrate is $\Delta A_{405nm} = (A_2 - A_{2C}) - (A_1 - A_{1C})$ or (A2 – A1), if no trypsin inhibitor control was run.
Note: It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range.

5. **Calculation:** Subtract 0 Standard from all readings. Plot the *p*-NA standard Curve. Apply the ΔA_{405nm} to the standard curve to get the nmol of *p*-NA (amount generated between T_1 and T_2 in the reaction wells).

Trypsin Activity = $\frac{B}{(T2-T1)\times V}$ × **Sample Dilution Factor = nmol/min/ml = mU/ml**

Where: **B** is the *p*-NA calculated from the Standard Curve (in nmol).
T_1 and **T_2** 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.

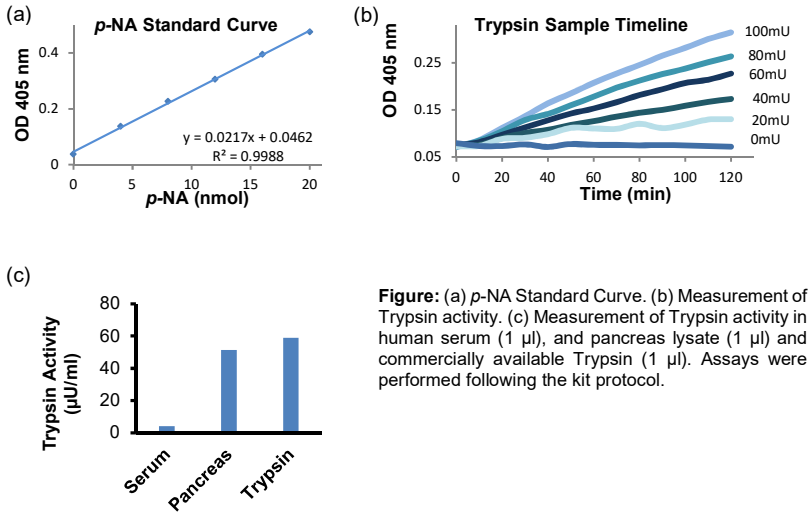

Figure: (a) *p*-NA Standard Curve. (b) Measurement of Trypsin activity. (c) Measurement of Trypsin activity in human serum (1 µl), and pancreas lysate (1 µl) and commercially available Trypsin (1 µl). Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

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

GENERAL TROUBLESHOOTING GUIDE: