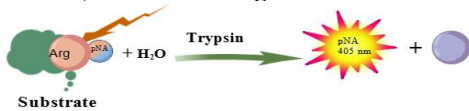


Trypsin Activity Colorimetric Assay Catalog: TBS2063 (100 Assays, Store at -20°C)

DESCRIPTION

Trypsin is produced in the pancreas as the inactive proenzyme trypsinogen. This enzyme plays a crucial role in protein digestion and in various physiological processes. The enzyme is very useful in protein research and clinical settings because it can be used to assess protein digestion, regulate cell signaling pathways, and diagnose conditions like acute pancreatitis.

Tribioscience Trypsin Activity Colorimetric Assay provides a simple and sensitive method for monitoring trypsin activity in biological samples (tissue, cells, serum, urine, stool). This assay uses a synthetic p- nitroaniline derivative (R-pNA) as its substrate. Trypsin can specifically cleave the substrate to release pNA, a chromophore which can be measured at absorbance (OD 405 nm) as shown in Fig. 1.



Synonyms: Trypsin; Trypsinogen.

APPLICATIONS

This kit is used for determination of trypsin activity in biological samples.

KEY FEATURES

Flexible: can be used for 96 wells and 384 wells plate

Simple: Just one-step: add-incubate-read model

Time saving: a 30-minute reaction at 37°C

KIT CONTENTS

Component	100x Rxns
Trypsin Substrate (10x)	1.0 mL
Standard (10mM)	0.1 mL
Positive control (10x)	5 µL
Assay Buffer	12 ml
Stop buffer	12 ml

STORAGE CONDITIONS

The kit is shipped on ice and should be stored at -20°C for long-term storage. Shelf life of 12 months after receipt.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and assay buffer to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic

enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

Reagent Preparation:

- Equilibrate all components to the room temperature. If substrate has precipitation in bottle, please warm up to dissolve the precipitation.
- Briefly vortex or pipette up and down all components (positive control can't be vortex) to ensure fresh reconstitution.

Reaction Preparation:

1. Label tubes as #1 through #8 as below table 1.
2. Add 126 µL of Assay Buffer to Std1, and 75 µL to Std2 to 8.
3. Pipet 24 µL of 10 mM standard stock into Std#1. Then, make 2x series dilution in Std2 through 7 with addition of 75 µL. Std8 is 1x Assay Buffer alone **as a standard 0**. The standard concentration in tube 1 through 7 will be 1600, 800, 400, 200, 100, 50, 25 µM, **Tube#8 is Standard 0 as blank**.

Table 1:

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	126	75	75	75	75	75	75	75
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	24	75	75	75	75	75	75	0
Final Conc (µM)	1600	800	400	200	100	50	25	0

4. Add 45 ul of assay buffer to the 10x positive control.
5. Dilute substrate stock (10x) with assay buffer for 10 times. For 96 well plate: 7.65 mL Assay buffer + 0.85 mL substrate stock (10x). Mix well gently.
6. Transfer 20 µL of each sample, blank, positive control, and standards into two separate wells.
7. Add 80 µL of the substrate solution to all samples, positive control, and blank wells. Add 80 µL of **Assay Buffer** to each **standard** wells (*Note: Do not add substrate in the standard*). Tap plate briefly to mix.
8. Incubate at 37°C for 30 minutes. Add 100 ul of stop solution in each well.
9. Read plate at OD 405nm in the endpoint mode.

CALCULATION

Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope, and use the following equation to calculate trypsin activity:

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Trypsin Activity (U/L) = DF * (ODSAMPLE – OD BLANK) / (t * Slope)

where ODSAMPLE is the OD405nm value for each sample and ODBLANK is the OD405nm value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 μmole of substrate to p-Nitrophenol per min at 37°C.

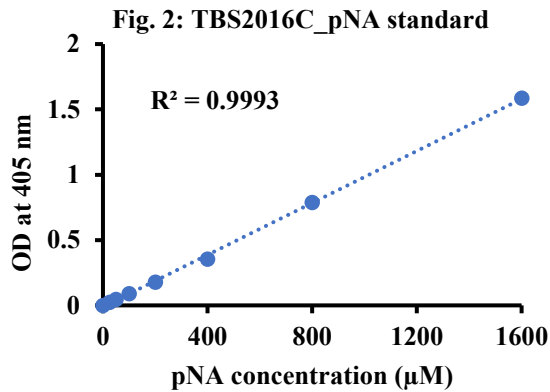
TYPICAL DATA

This standard curve is provided for demonstration only as below **Fig.2**. A standard curve should be generated for each set of samples assayed.

RELATED PRODUCTS:

- Caspase-3 Fluorometric Assay kit (TBS3230)
- Tryptase Activity Assay (TBS2101)
- Cytochrome C Oxidase Activity Assay (TBS2115)
- Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
- Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
- Non-esterified Fatty Acid Assay (TBS2203)
- Glycerol Colorimetric / Fluorometric Assay (TBS2204)
- Protein Assay Kits (TBS2005)
- Cell Nuclear Extract kit (TBS6025)

Research use only.



TYPICAL DATA of different concentrations of enzyme with substrate shown as Fig. 3.

