Colorimetric Trypsin Activity Assay (TRYP)

Catalog #8898

100 Tests in 96-well plate

Introduction

Trypsin is a member of the serine protease family. It cleaves proteins and peptides into smaller pieces by hydrolyzing peptide bonds at the carboxyl side of lysine and arginine residues. Trypsin is produced by the pancreas as an inactive trypsinogen and is then secreted into the small intestine, where it is cleaved by enteropeptidase and becomes activated. Trypsin activity aberration is implicated in gastrointestinal disorders such as pancreatitis and intestinal mucosal pathology. ScienCell's Colorimetric Trypsin Activity Assay Kit (TRYP) offers a rapid and sensitive way to determine the trypsin activity in mammalian cell/tissue lysates, serum, plasma and other biological fluid samples. Briefly, trypsin cleaves the substrate and releases p-nitroanilide (pNA), a chromophore that can be measured at 405 nm using a spectrophotometer. Chymotrypsin, another structurally similar serine protease, does not interfere with this assay because it does not cleave the trypsin substrate to release pNA.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8898a	1	Trypsin Assay Buffer (5X)	4 mL	4°C
8898b	1	Trypsin Substrate	500 µL	-80°C
8898c	1	Trypsin Positive Control	1 mL	-80°C
8898d	1	pNA Standard (5 mM)	400 µL	-80°C

Additional Materials Required (Materials Not Included in Kit)

Cell lysis buffer (e.g., 1X RIPA buffer)
96-well flat bottom plate
Plate reader or spectrophotometer

Product Use

TRYP is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, component #8898a should be stored at 4°C and #8898b, 8898c, and 8898d should be stored at -80°C. Protect from light. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary. If stored properly, the kit is good for up to 12 months.

Quality Control

Serially diluted Trypsin Positive Control samples (Cat #8898c) are measured using this kit.

Procedure (for a 96-well plate)

A. Standard curve plotting using the pNA standard

Note: This kit provides sufficient reagents for plotting 10 standard curves.

1. In wells #1-7, prepare the pNA Standard (5mM, Cat #8898d) and ddH_2O as shown in the table below.

Well #	1	2	3	4	5	6	7
5 mM pNA standard (µL)	0	1	2	3	4	5	6
ddH_2O (µL)	10	9	8	7	6	5	4
Amount of pNA in well (nmoles)	0	5	10	15	20	25	30

Note: Refreeze unused pNA Standard (Cat #8898d) to -80°C immediately after use. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary.

2. Add 20 µL of the Trypsin Assay Buffer (5X, Cat #8898a) and 70 µL of ddH_2O to 7 wells #1-7 and mix thoroughly by pipetting.
3. Read samples absorbance at 405 nm on a plate reader or a spectrophotometer. Calculate $\Delta OD_{405\ nm,\ \#n}$ of each pNA Standard sample.

$$\Delta OD_{405\ nm,\ \#n} = OD_{405\ nm,\ well\ \#n} - OD_{405\ nm,\ well\ \#1}$$

4. Create the standard curve by plotting $\Delta OD_{405\ nm}$ versus the pNA standard amount and derive the resulting standard curve equation: y=ax (see Figure 1 for an example of the standard curve).

   y is the $\Delta OD_{405\ nm}$ value
   x is the pNA standard amount
   a is the standard curve slope

B. Working reagent preparation and measurements

1. Lyse cell pellet or tissue samples using cell lysis buffer or homogenization of your choice (materials not provided) according to supplier's protocol. Serum and biological fluid samples can be used directly.
2. In each reaction well, dilute test samples (10-200 µg of cell lysate per well) to a final volume of 75 µL with ddH_2O. For the positive control, take 5 µL of the Trypsin Positive Control (Cat #8898c), and add 70 µL of ddH_2O. For the negative control sample use 75 µL of ddH_2O.
3. Add 20 µL of the Trypsin Assay Buffer (5X, Cat #8898a) and 5 µL of the Trypsin Substrate (Cat #8898b) to each test sample reaction well and mix thoroughly by pipetting.

   Note: Refreeze unused Trypsin Substrate (Cat #8898b) and Trypsin Positive Control (Cat #8898c) to -80°C immediately after use. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary.

4. Read the initial absorbance at 405 nm of the test samples and the controls on a plate reader or a spectrophotometer.
5. Incubate at 25°C for 1 hour, and protect from light. Incubate longer (up to 3 hours) if the trypsin activity is low. Read the final absorbance at 405 nm of the test samples and the controls on a plate reader or a spectrophotometer. Record the incubation time in minutes.
6. Using the equations below, determine the pNA product amount (nmoles) in each test sample reaction well and trypsin activity (mU/mL) of the sample.

   Trypsin unit definition: One unit is defined as the amount of trypsin that cleaves 1 µmole of Trypsin Substrate (Cat #8898b), yielding 1 µmole of pNA per minute at 25°C.

$$\Delta OD_{405nm,\ sample} = OD_{405nm,\ sample,\ final} - OD_{405nm,\ sample,\ initial}$$

$\Delta OD_{405nm,\ negative\ control} = OD_{405nm,\ negative\ control,\ final} - OD_{405nm,\ negative\ control,\ initial}$

$\Delta\Delta OD_{405nm,\ sample} = \Delta OD_{405nm,\ sample} - \Delta OD_{405nm,\ negative\ control}$

pNA product amount $_{sample}$ (nmoles) = $\Delta\Delta OD_{405nm,\ sample}$ / ***a***

$$\text{Trypsin Activity (mU/mL)} = \frac{\text{pNA product amount (nmoles) X 1000}}{\text{Incubation time (min) X lysate amount in well (µL)}}$$

C. Example calculations

Note: This example shows how to calculate trypsin activity expressed in mU/mL following the plotting of a standard curve using the pNA Standard (Cat #8898d).

10 µL sample was added to the reaction well. Incubation time is 90 min. The $\Delta\Delta OD_{405\ nm}$ for the test sample is 0.093. The standard curve of the pNA standard is shown below:

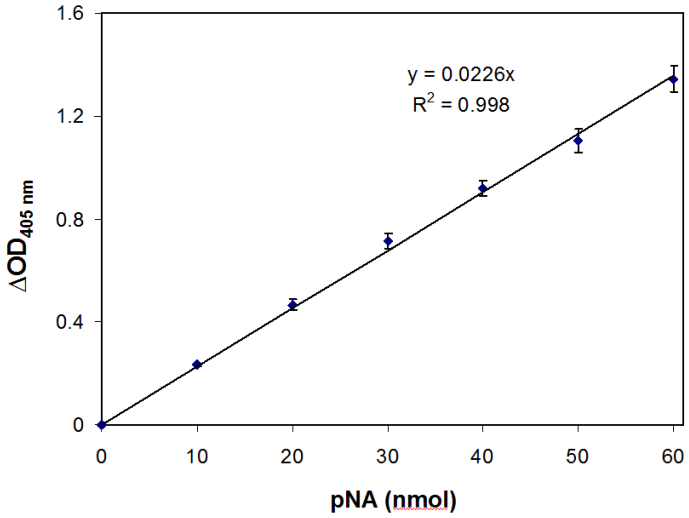

Figure 1. An example of pNA standard curve.

pNA product amount of test sample = (0.093/0.0226) nmoles = 4.12 nmoles

Trypsin activity of test sample = (4.12 nmoles x 1000) / (90 min x 10 µL)
= 46 nmoles/min/mL
= 46 mU/mL