Technical Bulletin

Trypsin Activity Assay Kit

Catalogue number MAK558

Product Description

Trypsin is a pancreatic serine protease, which hydrolyzes peptide bonds at the carboxyl side of arginine and lysine residues[1]. Trypsin is an important component of the process of protein digestion, by breaking large proteins into smaller peptides which are then further degraded into amino acids by other proteases. Trypsin is produced as the inactive trypsinogen in the pancreas which is activated by the enzyme enterokinase in the intestines. Trypsin itself can activate other proteases such as chymotrypsin and carboxypeptidase[2].

The Trypsin Activity Assay Kit is used for quantitively measuring Trypsin activity in a variety of samples, including tissues or cells. The assay is designed around the conversion of N-a-Benzoyl-Arginine-p-Nitroanilide (BAPNA) to p-NA and Benzoyl-Arginine by the enzyme trypsin. Detection of p-NA can then be read at 405 nm by a spectrophotometer.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer — 25 mL
  Catalogue Number MAK558A
- Trypsin Substrate — 0.2 mL
  Catalogue Number MAK558B
- Trypsin Positive Control — 1 Vial
  Catalogue Number MAK558C
- p-NA Standard — 0.4 mL
  Catalogue Number MAK558D
- Trypsin Inhibitor — 0.1 mL
  Catalogue Number MAK558E
- Chymotrypsin Inhibitor — 0.1 mL
  Catalogue Number MAK558F

Reagents and Equipment Required but Not Provided

- 96-well clear flat-bottom plate.
  (Catalogue number M2936 or equivalent)
- Plate reader that is capable to read absorbance at 405 nm.
- Pipettes
- Vortex Mixer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on dry ice. Store components at -20 °C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Avoid repeated freeze/thaw cycles.

Components MAK568B, MAK568D, MAK568E and MAK568F are dissolved in DMSO, which need to be warmed to room temperature before use.

Trypsin Positive Control - Reconstitute with 100 µL Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at –20 °C. Use within two months. Prevent freeze/thaw cycles.

Procedure

All Samples and Standards should be run in technical duplicates or triplicates.

Sample Preparation

Tissues or Cells:

1. Cut tissues to small pieces and homogenize in four-fold volume of cold assay buffer, by using a Dounce homogenizer or similar apparatus.
2. For cells, a concentration of 0.5-2 X 10^6 / mL of cells in four-fold volume cold assay buffer by using a Dounce homogenizer or similar apparatus.
3. Centrifuge samples at 10,000 RPM for 10 minutes.
4. Add the desired volume (2-25 µL) of supernatant to separate wells of a 96-well plate.
5. Add assay buffer to the sample wells to make a final volume of 50 µL per well.

Serum or Plasma samples may be used directly. Use assay buffer to adjust final volume to 50 µL per well.

Positive Control:

Add 5 µL of diluted MAK558C (at least by a factor of 10) to the well and add assay buffer to a final volume of 50 µL.

Inhibitor Control:

Add 1 µL trypsin inhibitor MAK558E to the desired sample and incubate for 5 minutes.

Preparation of Standards

A new standard curve should be prepared for every assay.

Add 0, 2, 4, 6, 8, and 10 µL of the p-NA standard directly in the 96-well plate and adjust volume to 50 µL using assay buffer as seen in Table 1.

Table 1.
Preparation of Standards

Tube	p-NA Standard (µL)	Assay Buffer (µL)	Concentration nmol/well
1	0	50	0
2	2	48	4
3	4	46	8
4	6	44	12
5	8	42	16
6	10	40	20

Preparation of Working Solution

Prepare 50 µL of working solution (WS) per well as follows:

2 µL Trypsin Substrate + 48 µL Assay Buffer

It is advised to make a single WS with enough solution to perform all tests for the run.

Assay Reaction

1. Add 50 µL of WS to each standard and sample well.
2. Incubate the plate protected from light at 25 °C for 10 minutes.
3. Read the initial absorbance at 405 nm (A_{405}) at T_0 (A_0 and A_{0C} for inhibitor control).
4. Incubate the plate at 25 °C for 1-2 hours (longer incubation times may be needed if trypsin activity is low).
5. Read the absorbance again (A_1 and A_{1c}). The change in absorbance, ΔA_{405}, is equal to: $(A_1 - A_{1c}) - (A_0 - A_{0c})$, or $(A_1 - A_0)$ if no inhibitor control was run.

Note: It is essential to read A_0 and A_1 in the reaction linear range. It is recommended to read the reaction kinetics for the complete assay duration, and then choose A_0 and A_1 that reside in the linear range.

Results

Calculations

1. Calculate the average value for each duplicate or triplicate of the standards and samples.
2. Subtract the blank (Standard 0) from all readings.
3. Plot the adjusted values of the p-NA standard curve.
4. Compare the ΔA405 to the standard curve to obtain the concentration of p NA (amount generated between T0 and T1 in the reaction wells).

$$\text{Trypsin Activity} = \frac{C \times D}{(T_1 - T_0) \times V}$$

Where:

nmole/min/mL = mU/mL

C = p-NA calculated from standard curve (nmole).

T_0 and T_1 = times of first and second readings (minute).

V = The volume (mL) of sample added to well.

D = sample dilution, if performed.

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

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

Figure 1.
Exemplary Standard Curve

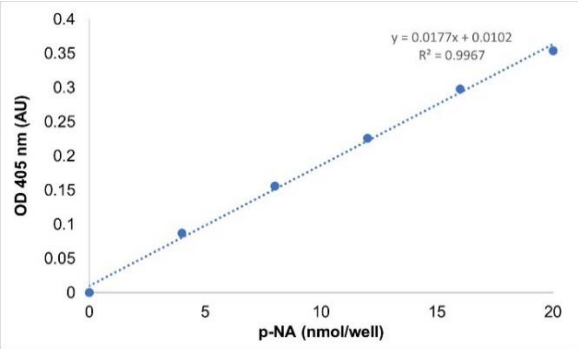
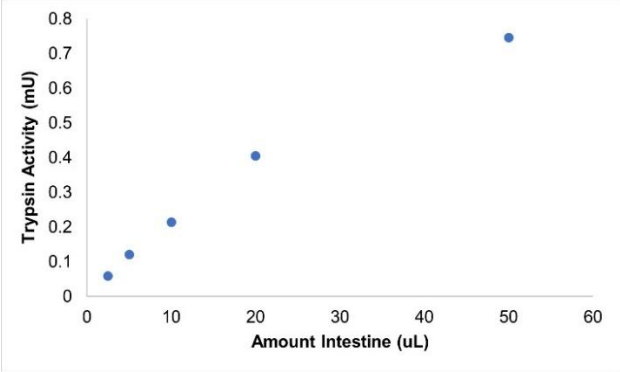


Figure 2.
Measurement of trypsin activity in various amounts of mouse intestines. Mouse intestines were homogenized in assay buffer supplemented with succinate and then centrifuged to collect the supernatant. Various amounts of the extract were assayed for 60 minutes using the kit components. The trypsin activity was calculated according to a p-NA standard curve, results in mU/well.



References

1. Guedidi, S. *et al*., enz. Microb. Tech. **51** (6–7), 325. (2012)
2. McDonald, J. K. *et al.,* Mammalian Proteinases: A Glossary and Bibliography, 2. FEBS LETTERS (1987)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader reads incorrect wavelength	Check settings of instrument
	Type of 96 well plate used	For colorimetric assays, use flat bottom, clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh reaction mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well/add 2-5 µL isopropyl alcohol to each well.
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin or use web calculator
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

Notice

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