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Product Information

Trypsin Activity Colorimetric Assay Kit

Catalog Number **MAK290**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Trypsin (EC 3.4.21.4) is a pancreatic serine protease, which hydrolyzes peptide bonds specifically at the carboxyl side of arginine and lysine residues. The rate of hydrolysis is slower if an acidic residue is on either side of the cleavage site and cleavage may not occur if a proline residue is on the carboxyl side. Tryptic digestion of the protein of interest results in a highly specific cleavage and a limited number of peptide fragments.

In this 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.

Components

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

Trypsin Assay Buffer Catalog Number MAK290A	25 mL
Trypsin Substrate (in DMSO) Catalog Number MAK290B	200 µL
Positive Control Catalog Number MAK290C	1 vial
p-NA Standard (2 mM) Catalog Number MAK290D	400 µL
Trypsin Inhibitor (TLCK, 20 mM) Catalog Number MAK290E	100 µL
Chymotrypsin Inhibitor (TPCK, 10 mM) Catalog Number MAK290F	100 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Trypsin Substrate, p-NA Standard, Trypsin Inhibitor, and Chymotrypsin Inhibitor – Are DMSO solutions, which need to be warmed up to room temperature to melt before use.

Positive Control – Dissolve with 100 µL of Assay Buffer. Pipette up and down to completely dissolve, aliquot, and store at –20 °C. Use within two months. Prevent freeze/thaw cycles.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>Standard Curve Preparations</u>

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

Sample and Positive Control Preparations

Tissues or cells can be extracted with 4 volumes of the Trypsin Assay Buffer and centrifuged in a micro-centrifuge at top speed for 10 minutes to obtain a clear extract. Bring the volume of the samples to 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. It is suggested to use several concentrations of the sample to ensure the readings are within the linear range.

Treat with 1 μL of 10 mM chymotrypsin inhibitor (TPCK) solution and incubate for 10 minutes at room temperature. For the positive control, add 5 μL of 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 20 mM trypsin inhibitor (TLCK) solution to trypsin inhibitor sample control and incubate for 5 minutes.

Reaction Mix Preparation

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μL of Reaction Mix containing 48 μL of Assay Buffer and 2 μL of 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.

Measurement

Initially measure absorbance at 405 nm (A_{405}) 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 $\Delta A_{405} = (A_2 - A_{2C}) - (A_1 - A_{1C})$ or $(A_2 - A_1)$, 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 to read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range.

Results

Calculation

Subtract 0 Standard from all readings. Plot the p-NA standard Curve. Compare the ΔA_{405} to the standard curve to obtain the nmole of p-NA (amount generated between T_1 and T_2 in the reaction wells).

$$\text{Trypsin Activity} = \frac{B}{(T_2 - T_1) \times V} \times DF$$

nmole/min/mL = mU/mL

B = *p*-NA calculated from the Standard Curve (nmole)
T_1 and T_2 = times of the first and second readings (minutes)
V = the pretreated sample volume added into the reaction well (mL)
DF= sample dilution factor

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.

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 at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	It is recommended to use clear plates.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	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 Mix 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 Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate 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
	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

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