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Product Information

Cathepsin B Inhibitor Screening Kit

Catalog Number **MAK200**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cathepsin B (CTSB; EC 3.4.22.1) is a lysosomal cysteine proteinase and is involved in intracellular degradation and turnover of proteins.[1] It is involved in the proteolytic processing of amyloid precursor protein (APP). Incomplete proteolytic processing of APP is a causative factor in Alzheimer's disease.[2] CTSB has also been implicated in the invasion and metastasis of certain cancers.[3]

The Cathepsin B Inhibitor Screening Kit is a simple assay for screening potential inhibitors of CTSB. CTSB activity is measured by cleaving a synthetic 7-amino-4-trifluoromethylcoumarin (AFC)-based substrate to yield AFC, a fluorescent product (λ_{ex} = 400/ λ_{em} = 505 nm), proportional to the enzymatic activity present.

Components

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

CTSB Reaction Buffer Catalog Number MAK200A	15 mL
CTSB Reagent Catalog Number MAK200B	100 µL
Cathepsin B, human Catalog Number MAK200C	1 vl
CTSB Substrate, Ac-RR-AFC, 10 mM Catalog Number MAK200D	0.2 mL
CTSB Inhibitor, F-F-FMK, 1 mM Catalog Number MAK200E	20 µL

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence 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

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

CTSB Reaction Buffer – Warm to room temperature before use.

CTSB Reagent – Aliquot and store at –20 °C.

Cathepsin B, human – Reconstitute with 110 µL of CTSB Reaction Buffer. Mix well by pipetting. Aliquot and store at –80 °C.

CTSB Substrate and CTSB Inhibitor – Ready to use. Store at –20 °C.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge the vials at low speed before opening.

Procedure

Sample Preparation

Prepare a 10× Sample Inhibitor Solution by diluting sample inhibitors with CTSB Reaction Buffer to 10× the final testing concentration. An initial concentrated inhibitor solution may be in a different solvent if the inhibitor is minimally soluble in aqueous CTSB Reaction Buffer.

To correct for background in samples, include a Sample Blank by omitting the Cathepsin B. The Sample Blank readings can then be subtracted from the sample readings.

For unknown samples, it is suggested to test several sample dilutions.

Prepare an Enzyme Control (uninhibited enzyme) by using CTSB Reaction Buffer in place of sample inhibitor.

An Inhibitor Control may be prepared by diluting 1 µL of CTSB Inhibitor with 9 µL of CTSB Reaction Buffer.

Add 10 µL of sample inhibitor (10× Sample Inhibitor Solution), Sample Blank (10× Sample Inhibitor Solution), Enzyme Control (CTSB Reaction Buffer), or Inhibitor Control into duplicate wells of a 96 well plate.

Assay Reaction

1. Set up Inhibition Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Inhibition Reaction Mix is required for each reaction (well).

Table 1.
Inhibition Reaction Mixes

Reagent	Samples and Controls	Sample Blank
CTSB Reaction Buffer	48 µL	49 µL
CTSB Reagent	1 µL	1 µL
Cathepsin B	1 µL	–

2. Add 50 µL of the appropriate Inhibition Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at room temperature for 10–15 minutes. Protect the plate from light during the incubation.

3. Set up an Enzymatic Reaction Mix according to the scheme in Table 2. 40 µL of the Enzymatic Reaction Mix is required for each reaction (well).

Table 2.
Enzymatic Reaction Mix

Reagent	Samples, Controls, and Sample Blank
CTSB Reaction Buffer	38 µL
CTSB Substrate	2 µL

4. Add 40 µL of the Enzymatic Reaction Mix to each reaction well. Mix well using a horizontal shaker or by pipetting.

5. Measure the fluorescence (FLU, λ_{ex} = 400/ λ_{em} = 505 nm) in a microplate reader in kinetic mode for 30–60 minutes at 37 °C. It is recommended to take fluorescent readings every minute.

Results

Calculations

Plot the fluorescence (FLU) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and obtain the slope for each well between T1 and T2. Determine the FLU at each time (FLU1 and FLU2) and use them to determine the slope of the plot (∆FLU/minute).

Note: The Enzymatic Control must be set up each time the assay is run.

Subtract the slope of the Sample Blank from the slope of the samples to obtain the corrected measurement. Use the corrected measurement to determine the % Relative Inhibition.

% Relative Inhibition

Slope = (FLU2 – FLU1)/(T2 – T1) = ∆FLU/minute

$$\% \text{ Relative Inhibition} = \frac{(\text{Slope}_{EC} - \text{Slope}_{SM})}{\text{Slope}_{EC}} \times 100\%$$

where:
Slope_{SM} = the slope of the Sample Inhibitor
Slope_{EC} = the slope of the Enzyme Control

Note: Irreversible inhibitors that completely inhibit Cathepsin B activity will have ∆FLU = 0. The % Relative Inhibition will be 100%.

Sample Calculation

Slope_{SM} = 0.435 FLU/min
Slope_{EC} = 0.755 FLU/min

$$\% \text{ Relative Inhibition} = \frac{(0.755 - 0.435)}{0.755} \times 100\% = 42.4\%$$

References

1. Reiser, J. et al., Specialized roles for cysteine cathepsins in health and disease. J. Clin. Invest., **120**, 3421–3431 (2010).
2. Cho, K. et al., CA-074Me, a cathepsin B inhibitor, decreases APP accumulation and protects primary rat cortical neurons treated with okadaic acid. Neurosci. Lett., **548**, 222–227 (2013).
3. Bao, W., et al., Silencing of Cathepsin B suppresses the proliferation and invasion of endometrial cancer. Oncol. Rep., **30**, 723–730 (2013).

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	For Fluorometric assays, use black plates with clear bottoms
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 needed to use 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 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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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