



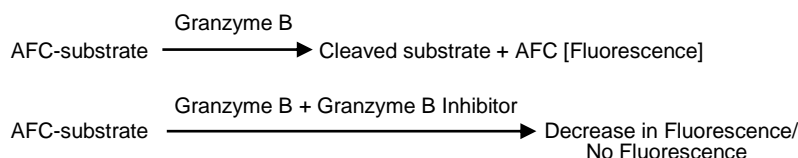
Granzyme B Inhibitor Screening Kit (Fluorometric)

rev.6/20

(Catalog # K169-100; 100 assays; Store at -20°C)

I. Introduction:

Granzyme B (GZMB, EC number 3.4.21.79), also known as Granzyme-2 is a serine protease stored in the granules of activated cytotoxic T cells and NK cells. Upon contacting with the target cells, Granzyme B is directionally exocytosed and with the assistance of perforin enters the target cells. With its unique substrate specificity (Granzyme B prefers an aspartic acid residue at the P1 site of its substrates), Granzyme B processes and activates various pro-caspases thereby inducing apoptosis in the target cells. In **BioVision's Granzyme B Inhibitor Screening Kit**, we have utilized a peptide substrate containing the Granzyme B recognition sequence along with a fluorescent label 'AFC'. Granzyme B catalyzes the cleavage of this substrate and releases the AFC molecule, which can be detected fluorometrically (Ex/Em = 380/500 nm). In the presence of potent Granzyme B Inhibitor, the hydrolyzation of substrate will be impeded. The Kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput screening of Granzyme B inhibitors or characterize/study Granzyme B inhibitors. Inhibitor control, Ac-IEPD-CHO is included to compare the efficacy of test inhibitors.



II. Applications:

- Screening Granzyme B inhibitors.
- Characterize/Study Granzyme B inhibitors.

III. Kit Contents:

Components	K169-100	Cap Code	Part Number
Granzyme B Assay Buffer	25 ml	WM	K169-100-1
Granzyme B Substrate	500 µl	Red	K169-100-2
Granzyme B Enzyme (human recombinant)	1 vial	Green	K169-100-3
Inhibitor Control (Ac-IEPD-CHO, 250 µM)	20 µl	Blue	K169-100-4

IV. User Supplied Reagents and Equipments:

- 96-well white plate with flat bottom
- Fluorescence microplate reader

V. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay.

VI. Reagent Preparation:

Granzyme B Enzyme: Reconstitute Granzyme B Enzyme with 220 µl Granzyme B Assay Buffer. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within one month.

VII. Granzyme B Inhibitor Screening Assay Protocol:

1. Enzyme Preparation: For each well, prepare 50 µl Granzyme B Enzyme solution.

48 µl Granzyme B Assay Buffer
2 µl Granzyme B Enzyme

Mix well. Add 50 µl of the Granzyme B Enzyme solution to each well.

2. Screen Compounds, Inhibitor Control and Blank Control Preparation:

Dissolve candidate inhibitors into proper solvent. Dilute to 4X the desired test concentration with Granzyme B Assay Buffer. Dilute desired volume of Inhibitor Control 1:25 with Granzyme B Assay Buffer. Add 25 µl diluted test inhibitors, diluted Inhibitor Control or Granzyme B Assay Buffer into Granzyme B Enzyme wells labeled as sample [S], Inhibitor Control (Ac-IEPD-CHO), or Enzyme Control [EC] (no inhibitor). Mix well, and incubate for 5 min.

3. Substrate Preparation: For each well, prepare 25 µl Granzyme B Substrate solution.

20 µl Granzyme B Assay Buffer
5 µl Granzyme B Substrate

Mix and add 25 µl Granzyme B Substrate solution into each well. Mix well.4.

4. Measurement: Incubate for 30-60 min at 37 °C, protected from light. Measure fluorescence at Ex/Em = 380/500 nm.

Note: We recommend measuring RFU in a kinetic mode and choose R₁ and R₂ at two time points (T₁ & T₂) in the linear range.

5. Calculation: The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta\text{RFU} = R_2 - R_1$. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate the relative % inhibition of the test inhibitors as:



$$\% \text{ Inhibition} = \frac{\Delta \text{RFU of EC} - \Delta \text{RFU of S}}{\Delta \text{RFU of EC}} \times 100\%$$

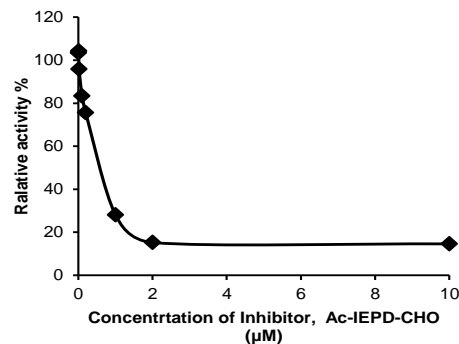


Figure: Inhibition of Granzyme B Activity by Granzyme B Inhibitor, Ac-IEPD-CHO. Assay was performed following the kit protocol.

VIII. RELATED PRODUCTS:

Human Calpain 1 Inhibitor Screening Kit
Granzyme A, human recombinant
Granzyme B, human recombinant
Granzyme B, mouse recombinant
Granzyme B, Antibody & Blocking Peptide
Granzyme B, Inhibitor Z-AAD-CMK & Ac-IEPD-CHO
Granzyme B Inhibitor Screening Kit
Caspase-1 Fluorometric Assay Kit
Caspase-2 Fluorometric Assay Kit
Caspase-3 Fluorometric Assay Kit
Caspase-4 Fluorometric Assay Kit
Caspase-5 Fluorometric Assay Kit
Caspase-6 Fluorometric Assay Kit
Caspase-8 Fluorometric Assay Kit
Caspase-9 Fluorometric Assay Kit
Caspase-10 Fluorometric Assay Kit
Caspase-12 Fluorometric Assay Kit
Caspase-1 Colorimetric Assay Kit
Caspase-2 Colorimetric Assay Kit

Caspase-1 Inhibitor Drug Screening Kit
Caspase-2 Inhibitor Drug Screening Kit
Caspase-3 Inhibitor Drug Screening Kit
Caspase-4 Inhibitor Drug Screening Kit
Caspase-5 Inhibitor Drug Screening Kit
Caspase-6 Inhibitor Drug Screening Kit
Caspase-7 Inhibitor Drug Screening Kit
Caspase-8 Inhibitor Drug Screening Kit
Caspase-9 Inhibitor Drug Screening Kit
Caspase-10 Inhibitor Drug Screening Kit
Calpain Activity Assay Kit
Cathepsin D Inhibitor Screening Kit
Caspase-3 Colorimetric Assay Kit
Caspase-4 Colorimetric Assay Kit
Caspase-5 Colorimetric Assay Kit
Caspase-6 Colorimetric Assay Kit
Caspase-8 Colorimetric Assay Kit
Caspase-9 Colorimetric Assay Kit
Caspase-10 Colorimetric Assay Kit

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