

Granzyme B Activity Fluorometric Assay Kit

rev. 3/13

(Catalog # K168-100; 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 target cell contact, Granzyme B is directionally exocytosed and with the assistance of perforin enters the target cell. 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 cell. In BioVision's Granzyme B Activity Assay 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). This Assay Kit is rapid, reliable and sensitive and can detect Granzyme B activity in various biological samples.



II. Application:

- Measurement of Granzyme B activity in various biological samples or purified Granzyme B activity.
- Study & characterize Granzyme B inhibitors.

III. Kit Contents:

Components	K168-100	Cap Code	Part Number
Granzyme B Assay Buffer	25 ml	WM	K168-100-1
Granzyme B Substrate	0.5 ml	Red	K168-100-2
Positive Control (Granzyme B Enzyme, human recombinant)	1 vial	Green	K168-100-3
AFC Standard (1mM)	0.1 ml	Yellow	K168-100-4

IV. User Supplied Reagents and Equipments:

- 96-well plate with flat clear bottom. Black plates are preferred for fluorometric assays.
- Fluorescent 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 prior to opening.

VI. Reagent Preparation and Storage Conditions:

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

VII. Granzyme B activity assay protocol:

- Sample Preparation:** Add 1-50 µl sample per well. For Positive Control, add 2 µl of Positive Control into desired well(s). Adjust final volume to 50 µl with Granzyme B Assay Buffer.
- AFC Standard:** Dilute AFC Standard to 10 µM by adding 10 µl of 1 mM AFC Standard to 990 µl Granzyme B Assay Buffer. Add 0, 5, 10, 15, 20 and 25 µl of 10 µM (10 pmol/µl) AFC Standard into a series of wells in 96 well plate to generate 0, 50, 100, 150, 200 and 250 pmol/well of AFC Standard. Adjust final volume to 100 µl/well with Granzyme B Assay Buffer.
- Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Granzyme B Assay Buffer	45 µl
Granzyme B Substrate	5 µl

Add 50 µl of the Reaction Mix into each well containing Positive Control and test samples. Mix well.

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

Note: Incubation time depends on the Granzyme B activity in the samples. We recommend measuring OD in a kinetic mode, and choose two time points (T₁ & T₂) in the linear range to calculate the Granzyme B activity of the samples. The AFC Standard Curve can read in Endpoint mode (i.e., at the end of incubation time).

- Calculation:** Subtract 0 Standard reading from all readings. Plot the AFC Standard Curve. Calculate the Granzyme B activity of the test sample: $\Delta\text{RFU} = R_2 - R_1$. Apply ΔRFU to the AFC Standard Curve to get B pmol of AFC generated due to hydrolyzation of substrate by Granzyme B during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample Granzyme B Activity} = \frac{B}{(\Delta T) \times V} \times \text{Dilution Factor} = \text{pmol/min/ml} = \text{U/ml}$$

Where: **B** is the AFC amount from Standard Curve (pmol).

ΔT is the reaction time (min).

V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of Granzyme B is the amount of enzyme that hydrolyzes 1 pmol of Ac-IEPD-AFC per min at 37°C.

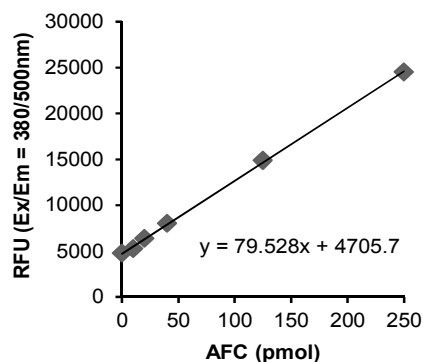


Figure: AFC Standard Curve.

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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