



.

Granzyme B Activity Fluorometric Assay Kit

rev 06/20

(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 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 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.

AFC-substrate -

Granzyme B Cleaved substrate + AFC [Fluorescence]

II. Applications:

- 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 white plate with flat bottom
- · 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 the small vials prior to opening.

VI. Reagent Preparation and Storage Conditions:

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

VII. Granzyme B activity assay protocol:

- 1. 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.
- 2. 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.
- 3. 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.

- 4. 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 RFU in 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).
- 5. Calculation: Subtract 0 Standard reading from all readings. Plot the AFC Standard Curve. Calculate the Granzyme B activity of the test sample: Δ RFU = R₂ – R₁. Apply Δ RFU to the AFC Standard Curve to get B pmol of AFC generated due to hydrolyzation of the substrate by Granzyme B during the reaction time ($\Delta T = T_2 - T_1$).

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

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

 $\Delta \mathbf{T}$ 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 µmol of Ac-IEPD-AFC per min at 37 °C.





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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