Product Information

Granzyme B Activity Fluorometric Assay Kit

I. Kit Contents:

Component	K2001-100 100 assays	Cap Color	Part Number
Granzyme B Assay Buffer	25 ml	WM	K2001-C-1
Granzyme B Substrate	0.5 ml	Red	K2001-C-2
Positive Control (Granzyme B Enzyme, human recombinant)	1 vial	Green	K2001-C-3
AFC Standard (1mM)	0.1 ml	Yellow	K2001-C-4

II. Introduction:

Granzyme B (GZMB, EC number 3.4.21.79) or Granzyme-2 is a serine protease located in the granules of activated cytotoxic T cells and Natural Killer cells. After target cell contact, Granzyme B is directionally exocytosed and enters the target cell which is facilitated by perforin. Granzyme B shows distinctive substrate specificity that prefers an aspartic acid residue at the P1 site of its substrates. It mediates and promotes different pro-caspases thus activating apoptosis in the target cells. This Granzyme B Activity Assay Kit can be used in different biological samples, it hydrolyzes the specific substrate to release the quench of fluorescent group, which can be detected fluorometrically at Ex/Em = 380/500 nm.

III. Reagent Preparation:

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

IV. 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:

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℃, 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).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the AFC Standard Curve. Calculate the Granzyme B activity of the test sample: ΔRFU = R2 - R1. 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 (ΔT = T2 - T1).

Sample Granzyme B Activity = B/((ΔT)×V) × Dilution Factor = pmol/min/ml = 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).

V. Storage and Stability:

Protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

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

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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