SIGMA-ALDRICH®
sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SensiZyme Granzyme B Activity Assay Kit

Catalog Number **CS1080**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Granzymes are exogenous serine proteases that are released from cytoplasmic granules within cytotoxic T cells (CTLs) and natural killer (NK) cells. Granzyme B is required for the rapid apoptotic signal delivered by CTLs and NK cells, an effect mediated by its ability to cleave and activate various procaspases. Granzyme B plays a crucial role in the development of acute graft-versus-host disease. Thus, the inhibition of this enzyme may prove to be an important tool in controlling immune responses. Conversely, the failure of Granzyme B induced apoptosis may contribute to certain pathologies. Such apoptosis is important for the elimination of virus-infected and malignant cells, and resistance to Granzyme B action (conferred by the serpin PI-9 proteinase inhibitor) may aid in the escape of some tumors from immune surveillance. Thus, Granzyme B plays a critical role in protecting organisms against intracellular infections and cellular transformation.[1]

The Granzyme B Activity Assay Kit provides all the reagents required for a highly sensitive detection of human Granzyme B activity in cell extracts, cell culture media, tissue extracts, body fluids (serum, plasma), and purified enzyme preparations. The kit was tested with the U-937 cell line and human leukocytes, serum, and plasma. A human Granzyme B Standard has been included for inhibitor screening.

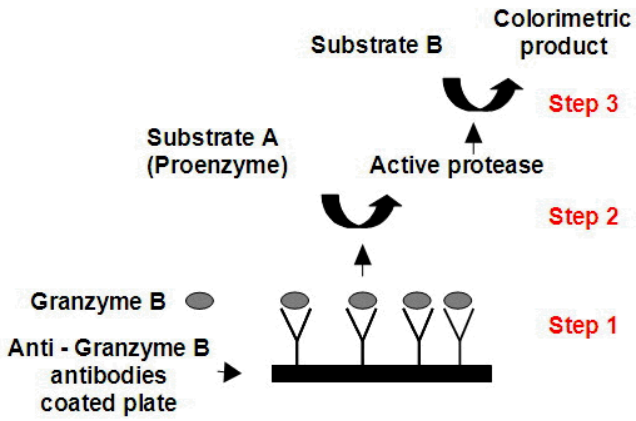
The protease activity measurement is based on a multistep series of reactions (see Figure 1).[2,3] In the kit assay, steps 2 and 3 are performed simultaneously.

Step 1: The Granzyme B containing extract is applied into a well, coated with a Granzyme B specific antibody (supplied with the kit).

Step 2: A modified protein substrate (Substrate A) is added to the well. Substrate A is a proenzyme containing the Granzyme B protease specific cleavage site fused to another protease. The proenzyme substrate is cleaved by Granzyme B to form an active "new" protease.

Step 3: A chromogenic peptide substrate (Substrate B) for the "new" protease is added to the well and is cleaved by the "new" protease. The change in the absorption of the chromogenic product is measured at 405 nm. The Granzyme B activity is directly proportional to the generation of color.

Figure 1.
Principle of Granzyme B Assay



This assay is sensitive and specific. The enhanced sensitivity is achieved by the signal amplification via the chain reaction. The specificity is achieved by both the immunochemical isolation of the Granzyme B enzyme from the extract by specific antibodies bound to the 96-well plate, and the use of an enzyme substrate (Substrate A) containing a Granzyme B specific cleavage site.

Components

The kit is sufficient for 96 reactions in the anti-Granzyme B coated 96-well plate.

Assay Buffer Catalog Number A5856	30 ml
Wash Buffer Catalog Number W4892	100 ml

Granzyme B Standard Catalog Number G2671	25 µl
Substrate A (proenzyme) Catalog Number S7197	300 µl
Substrate B Catalog Number S7322	1.5 ml
Anti-Granzyme B coated 96-well plate Catalog Number G2546	1 each

Equipment Needed but Not Provided
- Plate reader
- Humidified chamber
- Multichannel pipettor

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The Anti-Granzyme B coated 96-well plate (Catalog Number G2546) is composed of twelve 8-well strips. Before use, allow the anti-Granzyme B coated 96-well plate (the frame with the required number of strips) to warm to room temperature. Unused strips should be stored in a tightly closed nylon bag with a desiccant pack at 2–8 °C.

Before performing the assay, thaw the Wash buffer (Catalog Number W4892) and Assay Buffer (Catalog Number A5856) at room temperature, and Substrate A and Substrate B on ice. Ensure that the solutions are homogenous by gentle mixing.

Reaction Mixture – The Reaction Mixture should be freshly prepared before step 4 of the Procedure.
For each well/reaction prepare 100 µl of Reaction Mixture composed of:
- 82 µl of Assay Buffer (A5856)
- 3 µl of Substrate A (S7197)
- 15 µl of Substrate B (S7322)

For multiple reactions calculate the volumes required accordingly. Keep the prepared Reaction Mixture on ice until needed for the reaction. Store the remaining Substrate A and Substrate B at –20 °C and the Assay Buffer at 2–8 °C.

Standard Solutions - Just prior to beginning the assay, dilute an aliquot of the Granzyme B Standard (5 µg/ml, Catalog Number G2671) in Assay Buffer (Catalog Number A5856) according to Table 1. Mix well after each dilution. Store the Standard Solutions on ice until use. The Standard Solutions are used to determine a standard curve of Granzyme B activity.

Table 1.
Serial Dilutions of 5 µg/ml Granzyme B Standard

Standard sample	Granzyme B Standard (µl)	Assay Buffer (µl)	Granzyme B Standard final concentration (ng/ml)
1	2 (from 5 µg/ml)	998	10
2	250 (from 10 ng/ml)	250	5
3	250 (from 5 ng/ml)	250	2.5
4	250 (from 2.5 ng/ml)	250	1.25
5	250 (from 1.25 ng/ml)	250	0.625
6	250 (from 0.625 ng/ml)	250	0.312
7	250 (from 0.312 ng/ml)	250	0.156
8	250 (from 0.156 ng/ml)	250	0.078
9	250 (from 0.078 ng/ml)	250	0.039
10	250 (from 0.039 ng/ml)	250	0.02
11	250 (from 0.02 ng/ml)	250	0.01
12	250 (from 0.01 ng/ml)	250	0.005
Blank	0	250	0

Note: For detection of samples with high Granzyme B activity, use a standard curve consisting of standard samples 1-6 and the blank. For detection of samples with low Granzyme B activity, use a standard curve consisting of standard samples 6-12 and the blank.

Storage/Stability
The kit ships on dry ice and storage at –20 °C is recommended for all the components **except** the Anti-Granzyme B coated 96-well plate, which should be stored at 2–8 °C. Once thawed, the Assay Buffer and Wash Buffer should be stored at 2–8 °C, and it is recommended to store the Granzyme B Standard in aliquots at –70 °C. Avoid repeated freeze-thaw cycles.

Procedure

Granzyme B Activity Assay

When assaying multiple samples, coated wells and Substrate A should be from the same lot.

It is recommended to work in duplicates.

1. Pipette 100 µl of each standard and blank (see Table 1) separately into the appropriate well.
2. Pipette 100 µl of the test samples into other wells. Note: Samples with high Granzyme B activity should be diluted with Assay Buffer.
3. Cover the plate with the lid and incubate for one hour at room temperature.
4. Aspirate the solution from the wells and wash the wells 4 times with 200 µl each of Wash Buffer.
5. Blot the plate on tissue paper to remove any residual solution.
6. Pipette 100 µl of Reaction Mixture into each well. Cover the plate with the lid and incubate for 2–24 hours at 37 °C in a humidified chamber (i.e., a closed box with a source for humidity such as wet paper).
   Note: The incubation duration depends on the Granzyme B activity in the sample.
   - For Granzyme B concentrations in the range of 0.312–10 ng/ml, incubate the plate for 2–4 hours.
   - For Granzyme B concentrations in the range of 0.005–0.312 ng/ml, incubate for up to 24 hours.
7. Remove the lid and measure the absorbance at 405 nm using a plate reader
8. Calculate the sample activity using a standard curve.

Calculations

1. Calculate the average absorbance (of the duplicates) of the blank, each standard concentration, and the test sample. Subtract the average blank value from the average value of each standard and sample.
2. Plot the average absorbance of each standard concentration (y-axis) as a function of the Granzyme B concentration in the well (x-axis).

Results

Figures 2 and 3 show representative Standard Curves of Granzyme B activity at high or low Granzyme B concentrations, respectively. The difference in sensitivity is achieved by longer incubation periods.

Figure 2.

Standard Curve for Granzyme B (0.312–10 ng/ml)

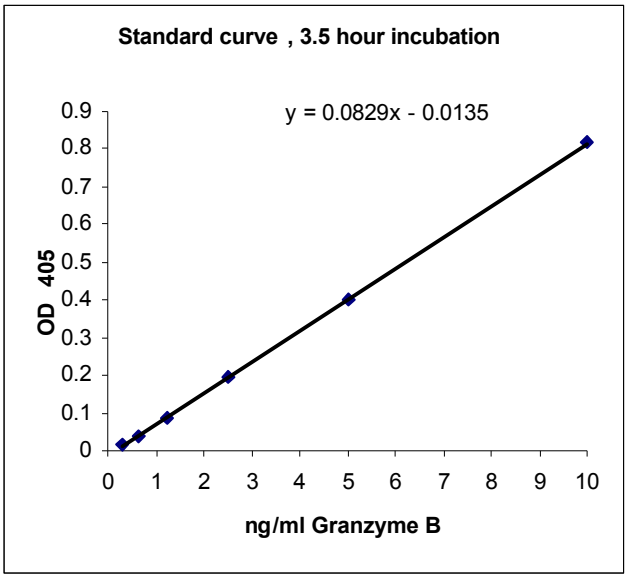
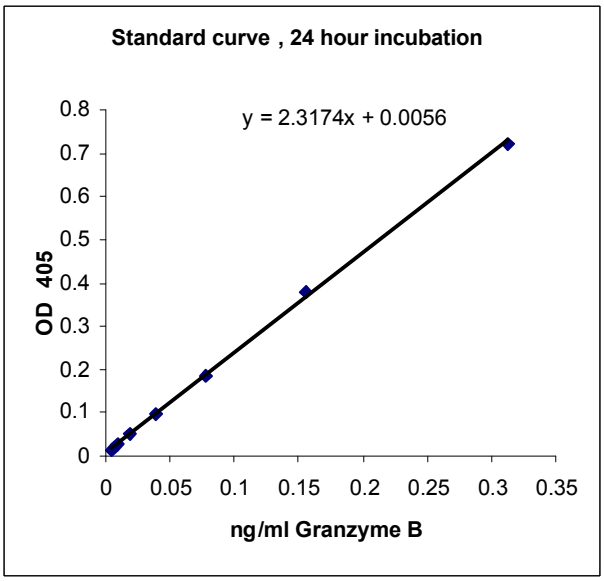


Figure 3.

Standard curve for low Granzyme B concentrations (0.005–0.312 ng/ml)



References

1. Caldas, H., et al., Survivin and Granzyme B–induced apoptosis, a novel anticancer therapy. *Mol. Cancer. Ther*., **5**, 693-703 (2006).
2. Verheijen, J.H., et al., Modified proenzymes as artificial substrates for proteolytic enzymes: colorimetric assay of bacterial collagenase and matrix metalloproteinase activity using modified pro-urokinase. *Biochem. J*., **323**, 603-609 (1997).
3. Verheijen, J.H., et al., Detection of a soluble form of BACE-1 in human cerebrospinal fluid by a sensitive activity assay. *Clin. Chem*., **52**, 1168-1174 (2006).

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