AssayGenie

For research use only

rev. 02/13

Calpain Activity Fluorometric Assay Kit

(Catalog #BN00508; 100 assays; Store kit at –70° C)

I. Introduction:

Activation of calpain is involved in many forms of physiological and pathological processes (e.g., apoptosis). Calpain activation requires cell membrane and Ca^{2+}, and activated calpain is released into cytosol. The **Calpain Activity Assay Kit** provides optimized buffers and reagents for a convenient measurement of calpain activity. The Extraction Buffer provided with the kit specifically extracts cytosolic proteins without contaminations of cell membrane and lysosome proteases. The Extraction Buffer also prevents auto-activation of calpain during the extraction procedure. Thus, the kit detects only activated calpain in cytosol upon treatment of cells with inducers (e.g., chemicals or drugs). The fluorometric assay is based on the detection of cleavage of calpain substrate Ac-LLY-AFC. Ac-LLY-AFC emits blue light (λ_{max} = 400 nm); upon cleavage of the substrate by calpain, free AFC emits a yellow-green fluorescence (λ_{max} = 505 nm), which can be quantified using a fluorometer or a fluorecence plate reader. Comparison of the fluorescence intensity from a treated sample with a normal control allows determination of the changes in calpain activity.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Extraction Buffer	25 ml	WM	BN00508-1
10X Reaction Buffer	1.5 ml	Clear	BN00508-2
Calpain Substrate Ac-LLY-AFC	0.5 ml	Amber	BN00508-3
Active Calpain I (Positive Control)	10 µl	Green	BN00508-4
Calpain Inhibitor Z-LLY-FMK	10 µl	Orange	BN00508-5

III. Calpain Assay Procedure:

1. Treat cells by desired methods. Concurrently incubate a control culture without treatment.
2. Count cells and pellet ~ 1 - 2 x 10^6 cells by centrifugation.
3. Resuspend cells in 100 µl Extraction Buffer and incubate samples on ice for 20 minutes. Gently mix the samples by tapping several times during incubation.
4. Centrifuge for 1 min in a microcentrifuge (10K x g) and transfer supernatant to a fresh tube and put on ice. Assay protein concentration (**Note: because of the high reducing agent content in the extraction buffer-dilute about 10-fold then use a Coomassie-based protein assay.**
5. Dilute the cell lysate (~ 50 - 200 µg) to 85 µl of Extraction Buffer.

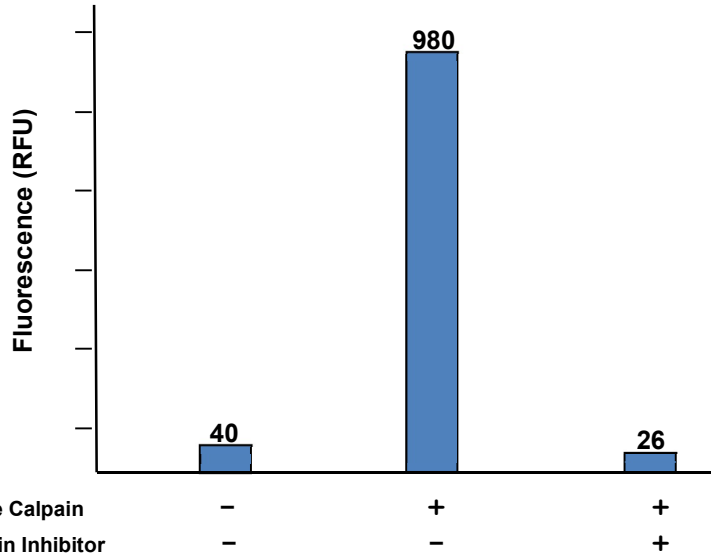
   For positive control, add 1 - 2 µl Active Calpain to 85 µl of Extraction Buffer.

   For negative control, use untreated cell lysate or add 1 µl Calpain Inhibitor to the treated cell lysate.
6. Add 10 µl of 10X Reaction Buffer and 5 µl of Calpain Substrate to each assay.
7. Incubate at 37° C for 1 hour in the dark.
8. Read samples in a fluorometer equipped with a 400 nm excitation filter and 505 nm emission filter. For a plate reading set up, transfer the samples to a 96-well plate.
9. The changes in calpain activity can be determined by comparing results of treated samples and negative control. Alternatively, the activity can be expressed as Relative Fluorescent Unit (RFU) per milligram protein of each sample.

FOR RESEARCH USE ONLY! Not to be used on humans.

IV. Storage and Stability:

Store kit at –70° C (Store Extraction Buffer and 10X Reaction Buffer at 4° C after opening). All reagents are stable for 6 months under proper storage conditions.



Typical Data for BN00510: Active Calpain (1 µg) was incubated at 37 °C for one hour using the Calpain Substrate (Ac-LLY-AFC) with or without 20 µM Calpain Inhibitor (Z-LLY-FMK)

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the substrate	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Substrate stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		