

ab39412

Caspase 1 Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of Caspase 1 activity in cell and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

INTRODUCTION

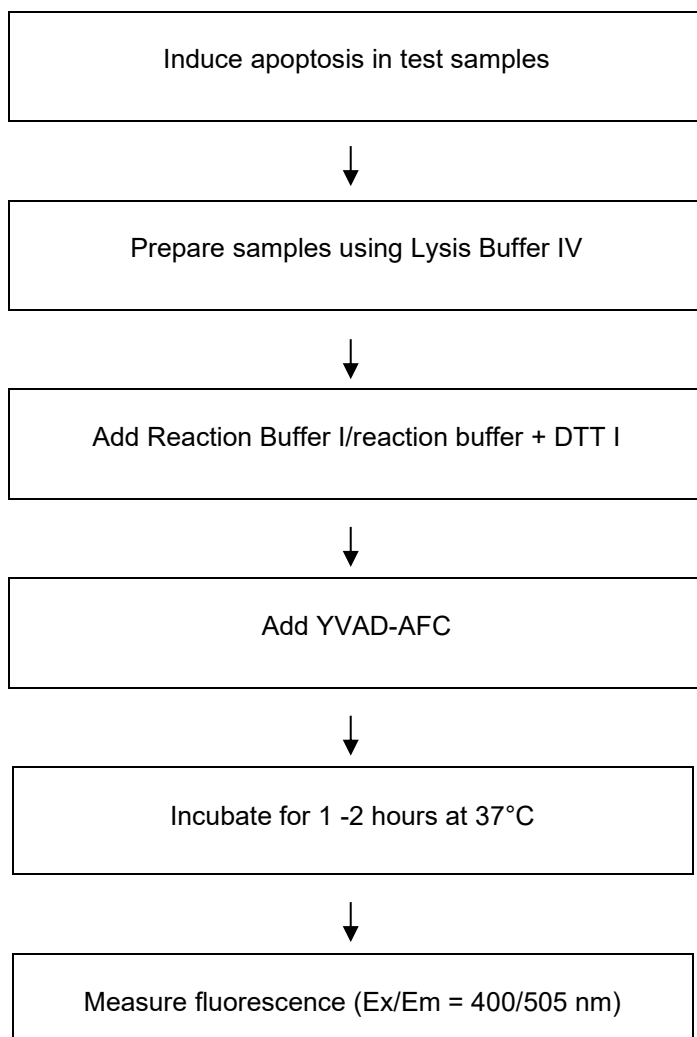
1. BACKGROUND	2
2. ASSAY SUMMARY	3
3. PRECAUTIONS	4
4. STORAGE AND STABILITY	4
5. MATERIALS SUPPLIED	5
6. MATERIALS REQUIRED, NOT SUPPLIED	5
7. LIMITATIONS	7
8. TECHNICAL HINTS	7
9. REAGENT PREPARATION	8
10. SAMPLE PREPARATION	9
11. ASSAY PROCEDURE AND DETECTION	11
12. CALCULATIONS	12
13. QUICK ASSAY PROCEDURE	13
14. FACTORS TO CONSIDER FOR CASPASE ACTIVITY ASSAYS	14
15. TROUBLESHOOTING	16
16. FAQ	18
17. INTERFERENCES	18
18. NOTES	19

1. BACKGROUND

Caspase-1 Assay Kit (Fluorometric) (ab39412) provides a simple and convenient method for detecting the activity of caspases that recognize the sequence YVAD. The assay is based on detection of cleavage of substrate YVAD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). The intact substrate YVAD-AFC emits blue light (λ max = 400 nm), and upon by caspase-1 or related caspases, the released free AFC molecule emits a yellow-green fluorescence (λ max = 505 nm), which can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from a treated sample with an untreated control allows determination of the fold increase in caspase-1 activity.

Caspase 1 (ICE, IL-1 β Converting Enzyme) is the prototypical member of the ICE family of proteases/caspases. Caspase 1 was first identified as a novel protease that generates the proinflammatory cytokine, interleukin 1 β (IL-1 β), by cleaving the pro-interleukin after the Asp116 residue. In addition to its role in the activation of proinflammatory cytokines, Caspase 1 also appears to have functions in some, but not all, types of apoptosis in mammalian cells.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 6 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Lysis Buffer IV	100 mL	-20°C	-20°C
2X Reaction Buffer I	4X 2 mL	-20°C	-20°C
YVAD-AFC	0.5 mL	-20°C	-20°C
DTT I (1M)	400 µL	-20°C	-20°C

PLEASE NOTE: 2X Reaction Buffer I was previously labelled as 2X Reaction Buffer, and Lysis Buffer IV as Cell Lysis Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 400/505 nm
- Flat bottom, opaque, white or black 96-well plates (black preferred).
- Black 96-well plates (clear bottoms) required for bottom-read
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)
- For information on how to produce a standard curve please see our resource page (<https://www.abcam.com/protocols/calculating-and-evaluating-elisa-data>) on the words resources page.
- Method for protein determination.

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample or control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Lysis Buffer IV:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **2X Reaction Buffer I:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **YVAD-AFC:**

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once thawed, use within 6 months. Keep on ice while in use.

9.4 **DTT I (1M):**

Ready to use as supplied. Aliquot DTT I so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed, use within 6 months.

10. SAMPLE PREPARATION

General Sample information:

- This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.
- We recommend performing several dilutions of your samples.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Treat samples with the desired method to induce caspase 1 activity. A common treatment to induce inflammation (and caspase 1) is addition of TNF alpha. Concurrently, incubate a separate culture without treatment to use as negative control.

10.1 Cell (adherent or suspension) samples:

- 10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = $1-5 \times 10^6$ cells).
- 10.1.2 Wash cells with cold PBS.
- 10.1.3 Resuspend cells in 50 μL of chilled Lysis Buffer IV.
- 10.1.4 Incubate on ice for 10 minutes.
- 10.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
- 10.1.6 Collect supernatant and transfer to a clean tube.
- 10.1.7 Keep on ice.
- 10.1.8 Optional: if protein concentration has been measured, use 50 – 200 μg cell lysate.

10.2 **Tissue samples:**

- 10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).
- 10.2.2 Wash tissue in cold PBS.
- 10.2.3 Resuspend tissue in 500 μ L of chilled Lysis Buffer IV.
- 10.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
- 10.2.5 Incubate on ice for 10 minutes.
- 10.2.6 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
- 10.2.7 Collect supernatant and transfer to a clean tube.
- 10.2.8 Keep on ice.
- 10.2.9 Optional: if protein concentration has been measured, use 50 – 200 μ g cell lysate.

11. ASSAY PROCEDURE AND DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all controls and samples in duplicate.

11.1 Set up Reaction wells:

- Sample wells = 1 – 50 μ L samples (or 50-200 μ g protein, adjust volume to 50 μ L/well with Lysis Buffer).
Use non-induced and induced samples.
- Background wells = 50 μ L Lysis Buffer IV (2 wells) and sample lysate only (2 wells).
- (Optional) Positive Control wells = 50 μ L Lysis Buffer IV + 1 – 2 U active caspase 1 enzyme.

11.2 Reaction Mix:

Prepare 50 μ L of Reaction Mix for each reaction.

Component	Reaction Mix (μ L)
2X Reaction Buffer I	49.5
DTT I	0.5

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the 2X Reaction Buffer I + DTT I to ensure consistency. We recommend the following calculation:

$X \mu\text{L component} \times (\text{Number samples} + 1)$.

- 11.3 Add 50 μ L of 2X Reaction Buffer I (containing 10mM DTT I) to each well.
- 11.4 Add 5 μ L of 1mM YVAD-AFC (50 μ M final concentration) to each well (do not add this to the sample lysate background wells).
- 11.5 Incubate at 37°C for 1-2 hours protected from light.
- 11.6 Measure output on a fluorescence microplate reader at Ex/Em = 400/505 nm.

12.CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the non-induced samples before calculating fold increase in caspase 1/ICE activity.
- Fold increase in Caspase-1 activity can be determined by comparing the results of induced samples with the level of the non-induced control.

13. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare 2X Reaction Buffer I, DTT I, substrate (aliquot if necessary); get equipment ready.
- (Optional) Prepare positive control.
- Prepare samples in duplicate.
- Set up plate for treated and untreated samples (50 μ L) and positive control (50 μ L).
- Prepare 2X Reaction Buffer I + DTT I (Number treated samples + untreated samples + positive control) + 1)

Component	Reaction Mix (μ L)
2X Reaction Buffer I	49.5
DTT I	0.5

- Add 50 μ L of 2X Reaction Buffer I + 10 mM DTT I to each sample.
- Add 5 μ L 1mM YVAD-AFC to all wells.
- Incubate at 37°C for 1-2 hours protected from light.
- Measure plate at Ex/Em= 400/505 nm.

14. FACTORS TO CONSIDER FOR CASPASE ACTIVITY ASSAYS

Three major factors need to be taken into account when using caspase activity assays:

- 18.1. The substrate in a particular assay is not necessarily specific to a particular caspase.
Cleavage specificities overlap so reliance on a single substrate/assay is not recommended. Other assays, such as Western blot, use of fluorescent substrates e.g. FRET assays should be used in combination with caspase activity assays.
- 18.2. The expression and abundance of each caspase in a particular cell type and cell line will vary.
- 18.3. As the activation and cleavage of caspases in the cascade will change over time, you should consider when particular caspase will be at its peak concentration e.g. after 3 hours, after 20 hours etc.

RESOURCES

The table below shows the known cross-reactivities with other caspases.

Inflammatory Caspases

Caspase	Cleavage motif	Inhibitor motif	Cross-reactivity with other caspase:									
			1	2	3	4	5	6	7	8	9	10
Caspase 1	YVAD					Y?	Y					
Caspase 4	LEVD	LEHD*					Y					
Caspase 5	WEHD	LEHD*	Y			Y						
Caspase 12	ATAD											

** inhibits at high concentration*

Classification of caspases based on synthetic substrate preference, does not reflect the real caspase substrate preference in vivo and may provide inaccurate information for discriminating amongst caspase activities. Thus, caution is advised in applying the intrinsic tetrapeptide preferences to predict the targets of individual caspases.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: Flat bottom, opaque, white or black 96-well plates (black preferred). Black 96-well plates (clear bottoms) required for bottom-read
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16.FAQ

We do not see any signal difference between the non-induced and induced samples. What may have gone wrong?

There can be multiple reasons for this. Freshly thawed DTT I needs to be added to the Reaction Buffer right before the experiment. The caspase induction conditions need to be optimized for dosage and time points for ideal detection. If possible, ensure caspase-1 induction by an alternate means as well (such as WB detection). Ensure that the YVAD-pNA is protected from light before use.

How can we control auto-activation during the lysis and assay procedure?

The Lysis Buffer IV will eventually lyse everything, however, only activated form can cleave the substrate. Autoactivation can be accounted for by using untreated samples as a control.

17.INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Protease inhibitors

18.NOTES



Technical Support

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