Cysteine Protease Activity Assay Kit

Catalog # 3101

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to assess cysteine protease activity
FORMAT:	96-well ELISA Plate with non-removeable strips
ASSAY TYPE:	Enzyme Assay/Fluorescence-based Assay
ASSAY TIME:	1 hour
STANDARD RANGE:	N/A
NUMBER OF SAMPLES:	Up to 42 (duplicate) samples/plate
SAMPLE TYPES:	Tissue homogenate, cell homogenate, culture media, or purified enzymes
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	Uses N-Succinyl-Leu-Leu-Val-Tyr-AMC (Read at Emission 380 nm/Excitation 460 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	All kit reagents must be warmed up to room temperature before running assay

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INTRODUCTION

Cysteine proteases are ubiquitous in all physiological aspects of living organisms (1). They are involved in a wide variety of processes such as immune responses, extracellular matrix management, apoptosis, etc. (2). Many cysteine proteases, such as Der p1 from house dust mite *Dermatophagoides pteronyssinus,* are synthesized as inactive zymogens with a proform to avoid unnecessary digestion and are later activated to gain proteolytic activity. (3,4)

The increased production and activity of certain cysteine proteases has been linked to diseases such as cancer or rheumatoid arthritis. (5). Evaluating cysteine protease levels is usually done with either ELISAs or cysteine protease activity assays. ELISAs do not distinguish between active and inactive forms of the enzyme and are better suited for determining total cysteine protease concentration. Cysteine protease activity assays will determine the level of cysteine protease activity, but depending on other factors present in samples, such as proteinase inhibitors or inactive zymogens, may underestimate the true cysteine protease level. (5). To accurately analyze cysteine protease activity in samples containing other proteinases, it may be necessary to add proteinase inhibitors to inactivate them.

Cysteine proteases may be good candidates for drug development if they can be targeted for their proforms to prevent maturation. (3) To assist such research, Chondrex, Inc. provides a fluorescent Cysteine Protease Activity Assay Kit (Cat # 3101) which uses a papain reference and a N-Succinyl-Leu-Leu-Val-Tyr-AMC substrate to measure cysteine protease enzyme activity in under an hour. Chondrex, Inc. also offers a Trypsin Activity Assay Kit (Cat # 3043) to evaluate serine protease activity and collagenase kits (Cat # 3001, 3002, 3014) to evaluate various matrix metalloproteinases. For more protease assay inquiries, please contact support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Papain Reference Standard (31011)	1 vial	25 units, Lyophilized	-20°C
N-Succinyl-Leu-Leu-Val-Tyr-AMC Substrate (31012)	1 vial	50 µl, 30 mM in DMSO	-20°C
Reference/Sample Dilution Buffer (31013)	1 bottle	50 ml	-20°C
Solution A (9052)	1 bottle	10 ml	-20°C
Stop Solution (31014)	1 bottle	10 ml	-20°C
96-Well ELISA Plate (Clear)	1 each	(Removable) 8-well strips x 12	-20°C
96-Well ELISA Plate (Black)	1 each	(Non-removable) 8-well strips x 12	-20°C

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NOTES BEFORE USING ASSAY

NOTE 1: Warm up all reagents to room temperature before use.

NOTE 2: It is recommended that the reference and samples be run in duplicate.

NOTE 3: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 4: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20°C for use in a future assay.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY OUTLINE

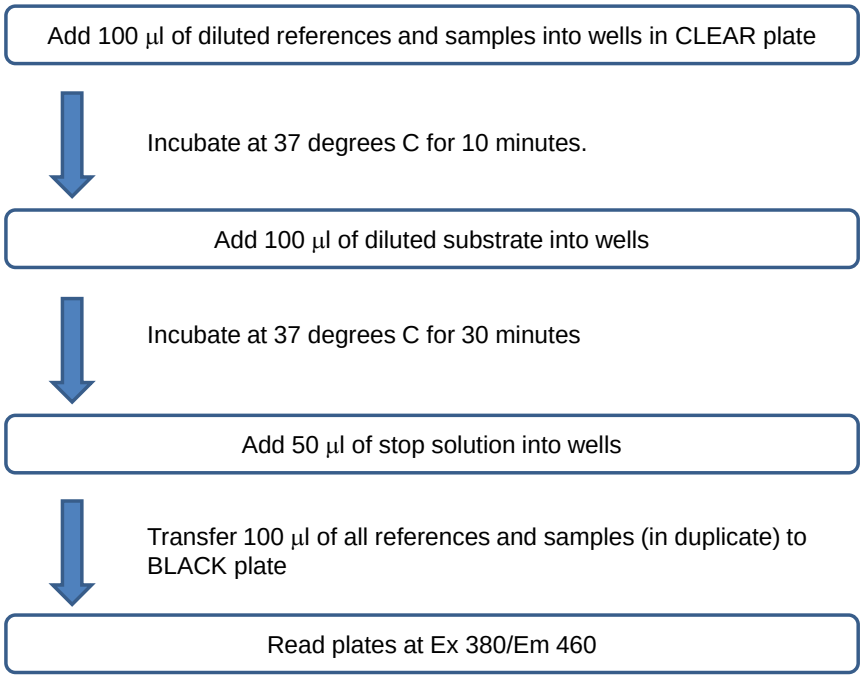

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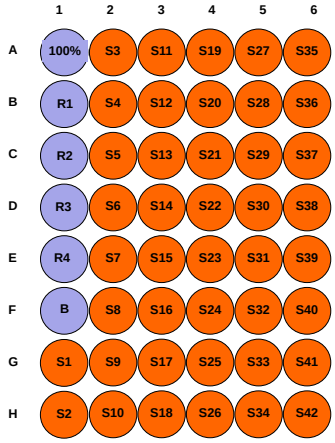
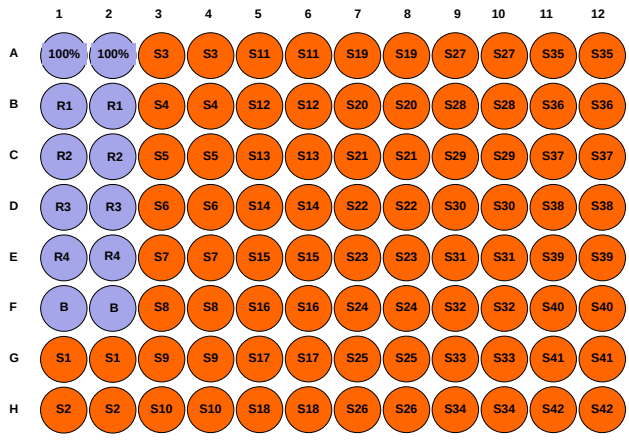
PLATE LAYOUT

Assay Layout (CLEAR plate)



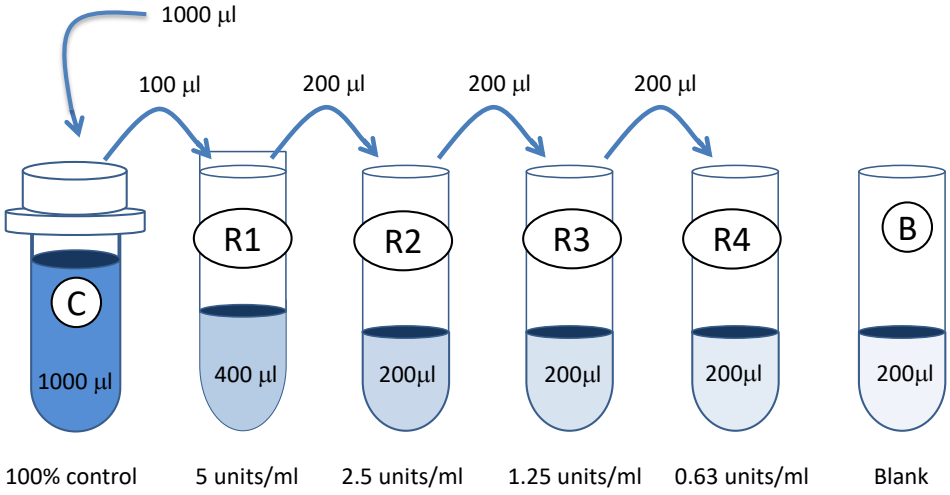
Read Layout (BLACK plate)



ASSAY PROCEDURE

1. **Prepare Reference Standard:** Four levels of reference standards are recommended. Dissolve 1 vial of Papain Reference Standard in 1 ml of Reference/Sample Dilution Buffer (100% control) and keep it as a reference stock and 100% control. Add 100 µl of this reference stock solution to 400 µl of dilution buffer to make a 5 units/ml solution (Reference 1). Then serially dilute it with Reference/Sample Dilution Buffer. For example, mix 200 µl of the 5 units/ml solution with an equal volume of Reference/Sample Dilution Buffer to make a 2.5 units/ml solution (Reference 2), and then repeat it two more times for 1.25 and 0.63 units/ml solutions (Reference 3 and 4, respectively). The remaining 100% control stock may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.

   NOTE: Evaluating colored samples such as cell culture media may reduce FI by approximately 5-10% due to quenching, thus the same culture media must be added to the 100% control tube for more accurate results. For more information about quenching and reference preparation, please contact support@chondrex.com.



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2. **Prepare Samples:** Samples can be tissue homogenates, cell homogenates, culture media, or purified enzymes. Centrifuge at 10,000 rpm for 5 minutes, then use the supernatant if the samples include insoluble materials. Dilute samples at least 1:1 with Reference/Sample Dilution Buffer depending on the estimated cysteine protease levels in the samples. Two to three different sample dilutions are recommended if the cysteine protease levels in the samples are unknown.

   NOTE 1: If the sample solution has color such as culture media, Chondrex, Inc. recommends adding such color to the 100% control in the reference standard preparation as indicated above.

   NOTE 2: Samples must be diluted with Reference/Sample Dilution Buffer to maintain optimal assay conditions.

   NOTE 3: Depending on the needs of the experiment, please consider appropriate protease inhibitors, especially cysteine protease inhibitors because they will affect the assay's outcome.

   NOTE 4: Freshly prepared samples are recommended for this assay. Samples can be immediately stored at -20°C after preparation and still be used for the assay. However, the enzyme activities will depend on the types of samples.

3. **Add 100% Control, References, and Samples to CLEAR plate:** Use the above assay plate layout for samples. Add 100 µl of the following into their respective wells: 100% control standard stock into the 100% control (100%) well, 5 – 0.63 units/ml References into the (R1-R4) wells, Reference/Sample Dilution Buffer into the (B) well, and samples into the orange wells.

4. **Incubate:** Cover the plate and incubate at 37 degrees C for 10 minutes.

5. **Prepare Substrate Dilutions:** Prepare the substrate solution with Solution A as shown in the following table (8 wells = 1 strip).

Strip #	Substrate (µl)	Solution A (ml)
1	9	0.9
2	17	1.7
3	25	2.5
4	33	3.3
5	42	4.2
6	50	5.0

6. **Add Substrate:** Add 100 µl of substrate solution to all wells.

7. **Incubate:** Cover the plate and incubate at 37 degrees C for 30 minutes.

8. **Add Stop Solution:** Add 50 µl of Stop Solution to all wells. Carefully tap the plate to mix.

9. **Transfer:** Carefully transfer 100 µl of all reference standards and samples (in duplicate) to the BLACK plate according to the above final plate layout.

10. **Read:** Read FI values of the BLACK plate at Excitation 360 nm and Emission 450 nm (apply auto-gain)

    NOTE: if FI values of samples are the same as the FI values of the 100% control, re-assay samples at a higher dilution.

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CALCULATING RESULTS

1. Average the duplicated FI values for blanks (B),100% control (C), references (R1-R4), and samples (S).

2. One unit of reference activity (1 unit) is defined as the cleavage of 1 nmole of substrate per minute (1 unit = 1 nmole/minute). Because this kit uses 30 nmole of substrate per well, activity and degradation are calculated using the following equations:

$$1 \text{ unit} = \frac{1 \text{ nmole}}{\text{Minutes}}$$

NOTE: The reference standard is used to check assay accuracy. Enzyme activity in individual samples must be calculated using the equations below.

Reference Degradation in 30 minutes (units)

$$\frac{(FI_{Sample} - FI_{Blank}) \times 30 \text{ nmole}}{(FI_{Control} - FI_{Blank}) \times 30 \text{ minutes}}$$

FI_{Blank}= FI in Blank

$FI_{Control}$= FI in 100% Control

FI_{Sample}= FI in Test Samples

Reference Activity in 30 minutes (units/ml)

$$\left(\frac{(FI_{Sample} - FI_{Blank}) \times 30 \text{ nmole}}{(FI_{Control} - FI_{Blank}) \times 30 \text{ minutes}} \right) \times \frac{1}{0.1 \text{ ml (per well)}}$$

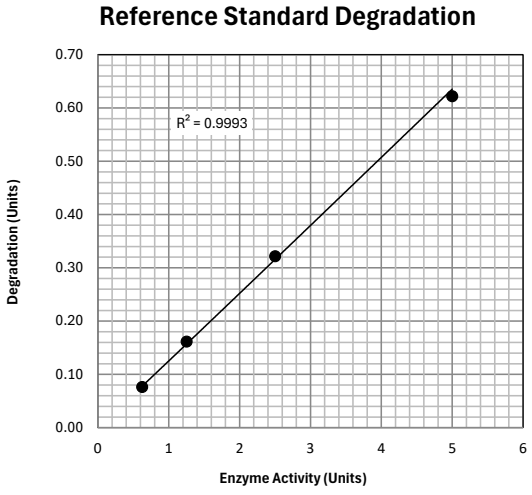
FI_{Blank}= FI in Blank

$FI_{Control}$= FI in 100% Control

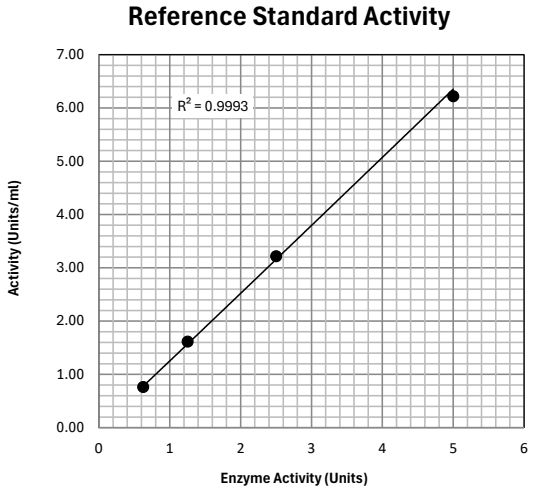
FI_{Sample}= FI in Test Samples

Figure 1 – Cysteine Protease Activity Assay Kit

Reference Standard Degradation at 30 minutes



Reference Standard Activity at 30 minutes



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s Assay FAQ for more information.

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