Trypsin Activity Assay Kit

Catalog 3043

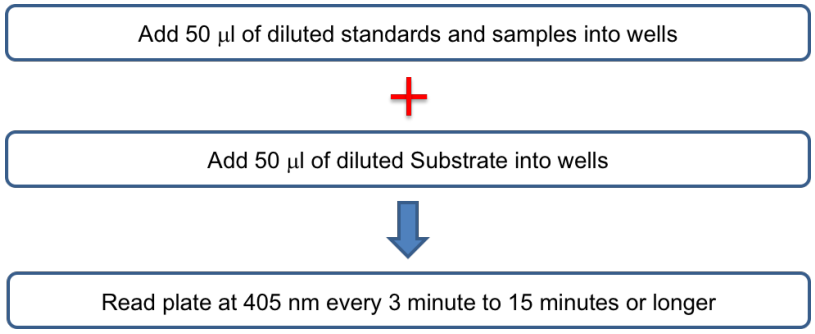
For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Trypsin is a mammalian serine protease and a member of the PA Clan (proteases of a mixed nucleophile, superfamily A), the largest of the cysteine and serine protease families. The PA Clan, which also includes trypsin-like proteases, can hydrolyze positively-charged amino acid peptide bonds in polypeptide chains, specifically the carbonyl group on Arg or Lys (1), effectively degrading peptides and proteins. As such, trypsin produced by the pancreas plays a key role in facilitating protein digestion and absorbance of foods in the small intestine. It also regulates the gastrointestinal immune response by controlling microbicide concentrations in the intestinal lumen and maintaining the integrity of the epithelial barrier (2-3). Due to these capabilities, trypsin is widely used in protein or peptide-related research for synthesizing and sequencing peptides, maintaining cultured cells, and digesting proteins (4). Recently, it was reported that proteinases in house dust mites promoted allergenicity to proteins, resulting in allergic reactions (5). This suggests that proteinases may play roles in the development of Type I allergies. In order to validate the study results, the ability to measure enzyme activity is critical.

Chondrex, Inc. introduces a chromogenic Trypsin Activity Assay Kit (Catalog # 3043) which uses a Boc-Gln-Ala-Arg-pNA substrate to measure trypsin-like enzyme activity in 15 minutes or more (up to 120 minutes, depending on the enzyme activity in samples). The trypsin cleaves the carbonyl group in the Arg of the substrate, liberating p-nitroanilid (pNA) which produces a yellow color and can be quantified using optical density (6). This kit works for trypsin and any proteinase/peptidase which cleaves the substrate. Therefore, to accurately analyze a specific proteinase's activity, it may be necessary to add proteinase inhibitors to inactivate other proteinases in samples. This kit, which employs a short assay time and a microplate format, is ideal for assaying many samples, as compared to traditional cuvette readings or gel assays (7). For more applications, please refer to published research references.

ASSAY OUTLINE



KIT COMPONENTS

Item	Quantity	Amount	Storage
Trypsin Reference Standard (30431)	1 vial	200 mUnits (pNA unit), Lyophilized	-20°C
Boc-Gln-Ala-Arg-pNA Substrate (30432)	1 vial	50 µl, 100 mM, in DMSO	-20°C
Solution B (67015)	1 bottle	50 ml	-20°C
96-well Microtiter Plate	1 each	96-well	Room temperature or-20°C

2607 151st Place NE
Redmond, WA 98052
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

NOTES BEFORE USING ASSAY

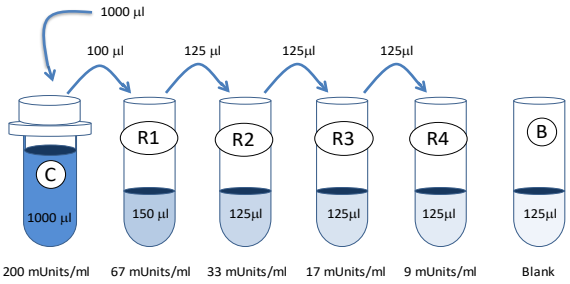
Note 1: It is recommended that the standards and samples be run in duplicate

Note 2: Warm up all reagents to room temperature before use

Note 3: Partially used reagents may be kept at -20°C

ASSAY PROCEDURE

1. **Prepare Trypsin References:** Four levels of reference standards are recommended. Dissolve 1 vial of Trypsin Reference Standard in 1 ml of Solution B (200 mUnits/ml) and keep it as a reference stock and 100% control. Add 100 µl of this standard stock solution to 150 µl of Solution B to make a 67 mUnits/ml solution (Reference 1). Then serially dilute it with Solution B. For example, mix 125 µl of the 67 mUnits/ml solution with an equal volume of Solution B to make a 33 mUnits/ml solution (Reference 2), and then repeat it two more times for 18 and 9 mUnits/ml solutions (Reference 3 and 4, respectively). The remaining 200 mUnits/ml reference stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



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 sample includes insoluble materials. Dilute samples at least 1:1 with Solution B depending on the estimated trypsin levels in the samples. Two to three different sample dilutions are recommended if the trypsin levels in the samples are unknown.

   Note 1: Samples must be diluted with Solution B to maintain optimal assay conditions.

   Note 2: If the sample solution has color such as culture media, we recommend using sample blank wells for the assays. Please refer to Step 3.

   Note 3: Depending on the needs of your experiment, use the appropriate protease inhibitors, especially trypsin 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.

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3. **Prepare Substrate Dilutions:** Prepare the substrate solution with Solution B as shown in the following table (16 wells = 2 strips).

Strip #	Substrate (µl)	Solution B (ml)
2	8	0.8
4	16	1.7
6	25	2.5
8	33	3.3
10	41	4.2
12	50	5.0

4. **Add 100% Control, Reference and Samples:**

Choose 4-1 or 4-2 depending on your samples.

4-1. Colorless Samples: Use the plate layout as shown in Figure 1A. Add 50 µl of the following into their respective wells: 200 mUnits/ml trypsin reference standard stock into the 100% control (C) wells, Solution B into the (B) wells, 67 - 9 mUnits/ml References into the (R1-R4) wells, and samples into the orange wells in duplicate. For example, add 50 µl of Sample 1 into the S1 wells, 50 µl of Sample 2 into the S2 wells, etc. Proceed to Step 5-1.

4-2. Colored Samples: Use the plate layout as shown in Figure 1B. Add 50 µl of the following into their respective wells: 200 mUnits/ml trypsin reference standard stock into the 100% control (C) wells, Solution B into the (B) wells, 67 - 9 mUnits/ml References into the (R1-R4) wells, and samples into the orange and gray wells in duplicate. For example, add 50 µl of Sample 1 into the S1 and SB1 wells, 50 µl of Sample 2 into the S2 and SB2 wells, etc. Proceed to Step 5-2.

Figure 1A - Standard Assay Layout (colorless samples)

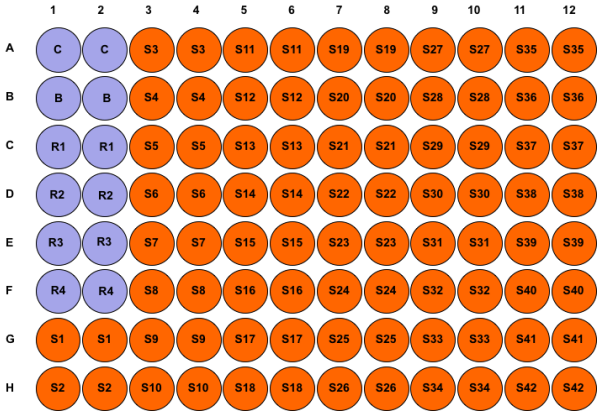
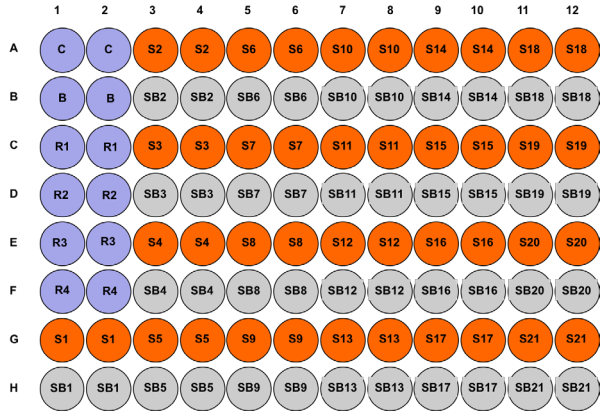


Figure 1B - Standard Assay Layout (colored samples)



5. **Add Substrate**

5-1. Colorless samples: Add 50 µl of substrate solution to all wells at room temperature (25°C).

5-2. Colored samples: Add 50 µl of substrate solution into the purple and orange wells and add 50 µl of Solution B into the gray wells at room temperature (25°C).

6. **Read:** If it is possible, keep the plate reader temperature at 25°C. Read the plate at 405 nm every 3 minutes for 15 minutes. If the trypsin activity is low, keep reading plate every 10 minutes, up to 120 minutes.

Note: If OD values of samples are the same as the OD values of the 100% control, dilute samples for another assay or adjust using kinetics analysis.

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CALCULATE TRYPSIN ACTIVITY

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

   Note: If you used sample blanks, the averaged OD values of the sample blanks (SB) are subtracted from the averaged OD values of the samples (S).

2. One unit of trypsin activity (1 pNA unit) is defined as the cleavage of 1 μmole of substrate per minute (1 Unit = 1 μmole/minute). Because this kit uses 5 μmole of substrate per assay, trypsin activity is calculated using the following equation:

   Note: one pNA Unit = 0.615 TAME Unit = 35 BAEE Unit.

Trypsin Activity at 15 Minutes (pNA units/ml) =

$$\frac{(OD_{Sample} - OD_{Blank}) \times 5\ \mu mole}{(OD_{Control} - OD_{Blank}) \times 15\ (minutes) \times Sample\ Volume\ (ml)}$$

OD_{Blank} = OD in blank (at 15 minutes)

$OD_{Control}$ = OD in 100% control (at 15 minutes)

OD_{Sample} = OD in test samples (at 15 minutes)

Note: The trypsin reference standard is used to check assay accuracy. Trypsin activity in individual samples must be calculated using the equation above.

OPTIONAL

If samples have high trypsin activity, then at the 15 minute point, the OD values may be the same as the 100% reference control. In this case, calculate enzyme activity using the following equation:

Trypsin Activity (pNA units/ml) =

$$\frac{(OD_{Sample\ time\ B} - OD_{Sample\ time\ A}) \times 5\ \mu mole}{(OD_{Control} - OD_{Blank}) \times \Delta Time\ (minutes) \times Sample\ Volume\ (ml)}$$

OD_{Blank} = OD in blank (at 15 minutes)

$OD_{Control}$ = OD in 100% control (at 15 minutes)

$OD_{Sample\ time\ A}$ = OD in test samples at Time A (ex: 6 minutes)

$OD_{Sample\ time\ B}$ = OD in test samples at Time B (ex: 9 minutes)

$\Delta Time$ = Time B - Time A

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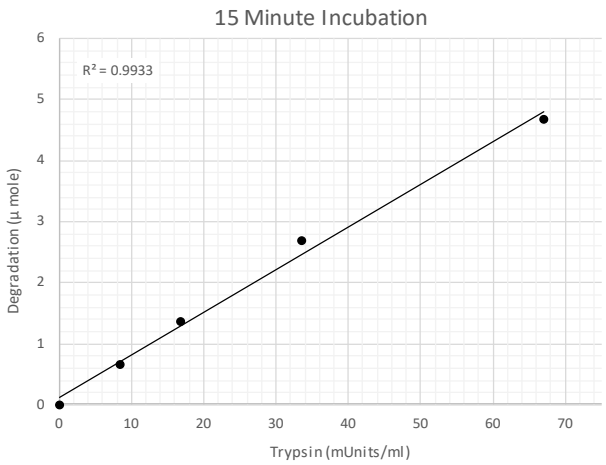
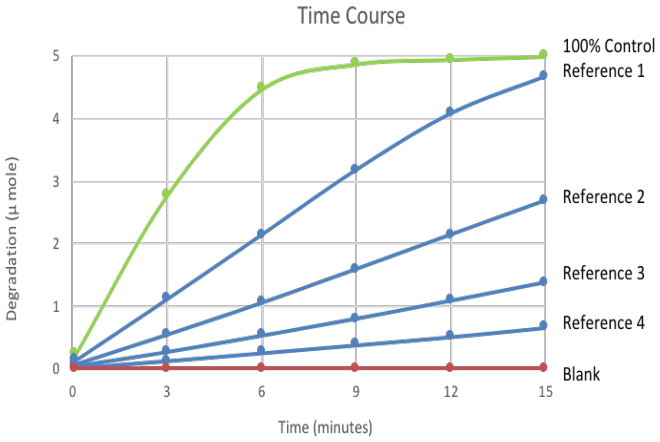
Figure 2 - Trypsin Activity

(a) Dose Response Curve



(b) Time Course



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