Trypsin Activity Assay Kit

Catalog # 3043

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to measure trypsin activity
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Chromogen Assay
ASSAY TIME:	15 to 120 minutes
STANDARD RANGE:	N/A
NUMBER OF SAMPLES:	Up to 42 (duplicate) colorless samples/plate and up to 21 (duplicate) colored samples/plate
SAMPLE TYPES:	Tissue homogenate, cell homogenate, culture media, or purified enzymes
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	Uses a Boc-Gln-Ala-Arg-pNA substrate (read at 405 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	N/A

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INTRODUCTION

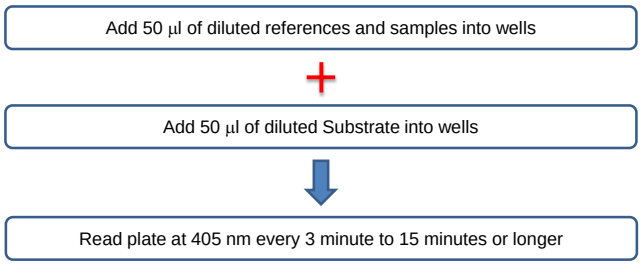
Trypsin is a mammalian serine protease and a member of the PA Clan (proteases of a mixed nucleophile, superfamily A), the largest of the cysteine and serine protease families. The PA Clan, which also includes trypsin-like proteases, can hydrolyze positively charged amino acid peptide bonds in polypeptide 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 (Cat # 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-nitroanilide (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 contact support@chondrex.com for published research references.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Trypsin Reference (30431)	1 vial	200 mUnits, 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 ELISA Plate	1 each	8-well strips x 12	-20°C

ASSAY OUTLINE



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NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

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

PLATE LAYOUT

Standard Assay Layout (colorless samples)

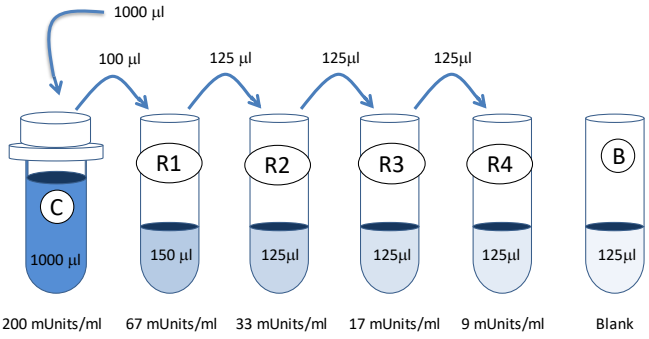
	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
B	B	B	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
C	R1	R1	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
D	R2	R2	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
E	R3	R3	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
F	R4	R4	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
G	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
H	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42

Standard Assay Layout (colored samples)

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18
B	B	B	SB2	SB2	SB6	SB6	SB10	SB10	SB14	SB14	SB18	SB18
C	R1	R1	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19
D	R2	R2	SB3	SB3	SB7	SB7	SB11	SB11	SB15	SB15	SB19	SB19
E	R3	R3	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20
F	R4	R4	SB4	SB4	SB8	SB8	SB12	SB12	SB16	SB16	SB20	SB20
G	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17	S21	S21
H	SB1	SB1	SB5	SB5	SB9	SB9	SB13	SB13	SB17	SB17	SB21	SB21

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. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



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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 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, Chondrex, Inc. recommends using sample blank wells for the assays. Please refer to Step 4.

   NOTE 3: Depending on the needs of the experiment, please consider 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.

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	17	1.7
6	25	2.5
8	33	3.3
10	42	4.2
12	50	5.0

4. **Add 100% Control, References, and Samples:** Choose 4-1 or 4-2 depending on samples

   4-1. Colorless Samples: Use the plate layout for colorless samples. 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 for colored samples. 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.

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 the 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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CALCULATING RESULTS

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

   NOTE: If using sample blanks, the averaged OD values of the sample blanks (SB) are subtracted from the averaged OD values of the samples (S).

2. One mUnit of trypsin activity (1 pNA mUnit) is defined as the cleavage of 1 nmole of substrate per minute (1 mUnit = 1 nmole/minute). Because this kit uses 50 nmole of substrate per well, trypsin activity is calculated using the following equation:

   NOTE: one pNA mUnit = 0.615 TAME mUnit = 35 BAEE mUnit.

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

$$\frac{(OD_{Sample} - OD_{Blank}) \times 50 \text{ nmole}}{(OD_{Control} - OD_{Blank}) \times 15 \text{ (minutes)} \times \text{Sample Volume (ml)}}$$

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 mUnits/ml) =

$$\frac{(OD_{Sample\ time\ B} - OD_{Sample\ time\ A}) \times 50 \text{ nmole}}{(OD_{Control} - OD_{Blank}) \times \Delta\text{Time (minutes)} \times \text{Sample Volume (ml)}}$$

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

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

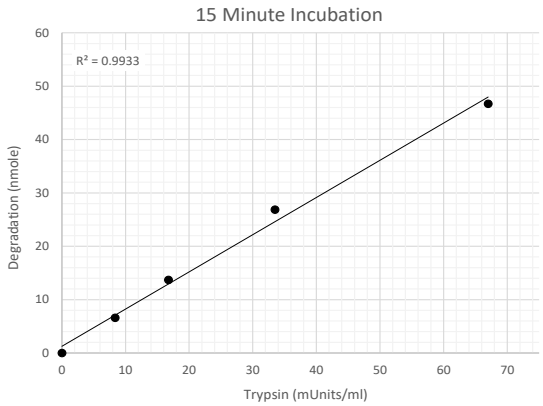
$OD_{Sample\ Time\ A}$ = OD in Test Samples at Time A (Ex: 6 minutes)

$OD_{Sample\ Time\ B}$ = OD in Test Samples at Time B (Ex: 9 minutes)

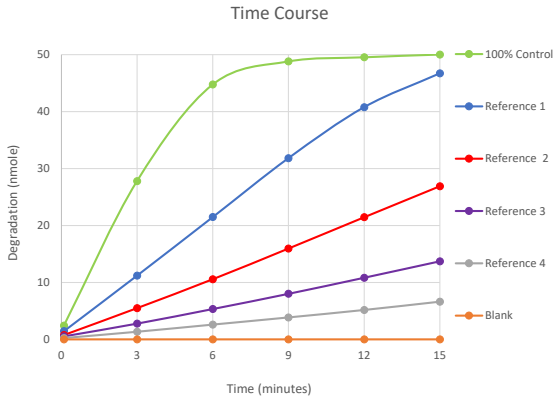
ΔTime = Time B – Time A

Figure 1 - Trypsin Activity Assay Kit

Dose Response Curve



Time Course



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TROUBLESHOOTING

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

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