MMP-13 Inhibitor Assay Kit

Catalog # 3089

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to assess inhibitory activity to MMP-13
FORMAT:	96-well ELISA Plate with non-removeable strips
ASSAY TYPE:	Enzyme Assay/Fluorescence-based Assay
ASSAY TIME:	1-1.5 hours
STANDARD RANGE:	Depends on incubation time
NUMBER OF SAMPLES:	Up to 44 (duplicate) samples/plate
SAMPLE TYPES:	Culture Media and Tissue Homogenate
RECOMMENDED SAMPLE DILUTIONS:	Depends on inhibitory activity in samples
CHROMOGEN:	N/A (Read at Emission 450 nm/Excitation 365 nm)
STORAGE:	-20°C for 12 months (Reference Standard is stored at -80°C)
VALIDATION DATA:	N/A
NOTES:	Uses fluorogenic peptide substrate

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION

MMP-13 (collagenase 3) is a matrix metalloproteinase found in various tissues such as malignant tumors, osteoarthritic cartilage, rheumatoid synovium, and wounds. MMP-13 production in chondrocytes and synoviocytes is upregulated by stimulation with inflammatory mediators such as IL-1, TNF and retinoic acid (1). MMP-13 has been shown to degrade type I and II collagen and the degradation of type II collagen occurs approximately ten times faster than that of type I collagen (2). The typical 3/4 and 1/4 fragments of collagen are produced as with MMP-1 (collagenase 1). However, MMP-13 also generates a second cleavage of type II collagen that removes three amino acids from the amino terminus of the 1/4 fragment. In addition, MMP-13 degrades aggrecan, the major proteoglycan of cartilage (3).

Inhibitors of MMPs may be useful therapeutics to prevent metastasis of certain cancer cells and tissue damage in inflammatory diseases. Chondrex, Inc. provides an MMP-13 Inhibitor Assay Kit (Cat # 3089), replacing obsolete Cat # 3003. This improved MMP-13 inhibitor assay kit has been optimized to be a faster, more reliable assay, ideal for assessing inhibitors of MMP-13 and related enzymes. MMP-13 inhibitors can be easily screened and evaluated by simply adding potential inhibitory compounds to the activated recombinant human MMP-13 (rhMMP-13). This kit contains a truncated rhMMP-13 that can cleave the kit's fluorogenic peptide substrate (Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH_2), but is not capable of digesting native collagen molecules.

Furthermore, this kit is useful for assaying total collagenase-like proteinase activity in tissue fluids or extracts and cell culture supernatants. Because the fluorogenic peptide substrate used in this kit does not hold a triple helical structure, this substrate is not specific to collagenase and might be susceptible to non-collagenolytic proteinases. Therefore, it is important to know the total activity of collagenase-like proteinases in specimens before proceeding with further experiments.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Reference: Recombinant Human MMP-13 (Truncated) (5003)	1 vial	100 µl, 100 µg/ml	-80°C
100X Substrate - Fluorogenic Peptide (30033)	1 vial	100 µl, 2 mM in DMSO	-20°C
Solution A - Substrate Dilution Buffer (30032)	1 bottle	10 ml	-20°C
Solution B - Sample Dilution and Reaction Buffer (30031)	1 bottle	50 ml	-20°C
Proteinase Inhibitor (30046)	1 vial	3 mg lyophilized	-20°C
Stop Solution - o-Phenanthroline (300410)	1 vial	1 ml, 10 mM in Ethanol	RT
Black ELISA Plate	2 each	96-well	RT

NOTES BEFORE USING ASSAY

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

NOTE 2: Most synthetic, organic compounds are water insoluble. Dissolving test compounds in water is required for screening and testing biological and pharmacological activities. Solvents such as alcohol, DMSO, PEG, and PPG may be used to dissolve water insoluble compounds. These test samples must then be diluted with the Sample Dilution and Reaction Buffer (Solution B) prior to incubation. The pH of the sample solution should be 7.5 - 8.0.

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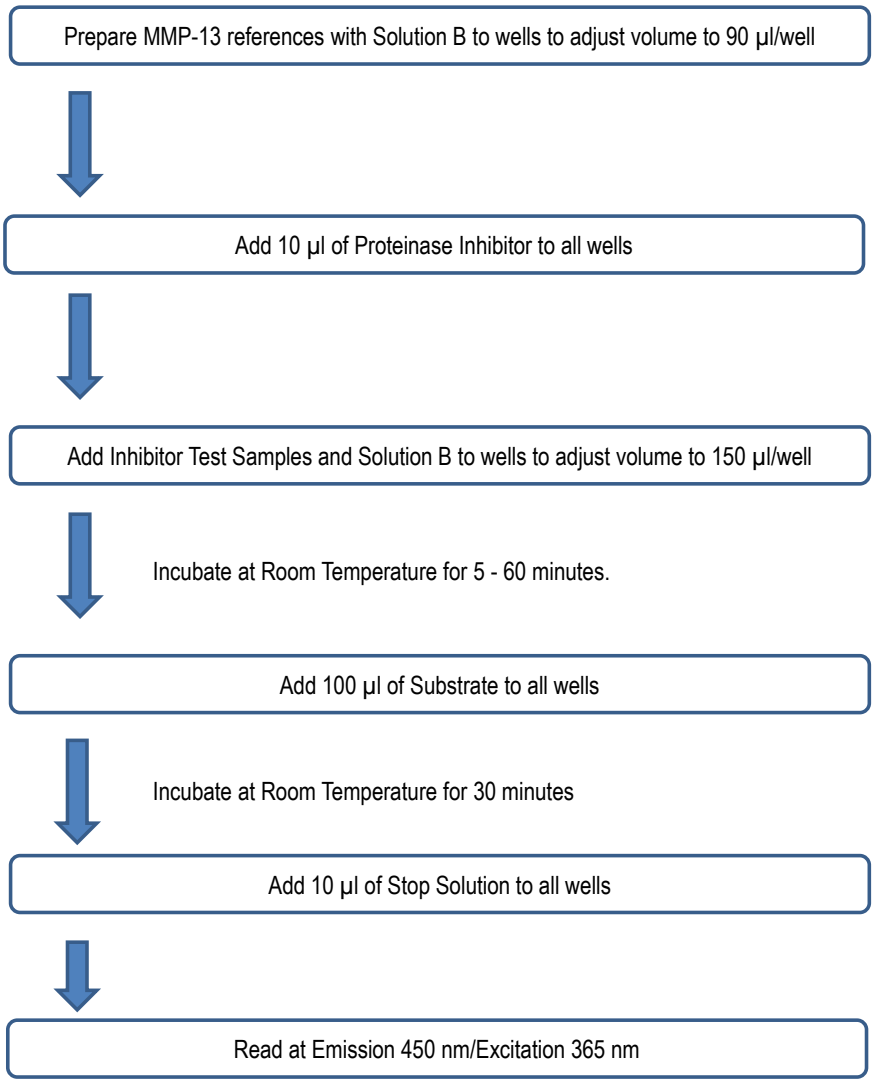
ASSAY OUTLINE



ASSAY PROCEDURE

1. **Prepare References and Samples**: Designate reactions to be performed in the black 96-well microtiter plate for blank, References, and samples. A sample layout is shown in the MMP-13 Inhibitor assay sheet at the end of this protocol. Dilute the reference rhMMP-13 (100 µg/ml) with Sample Dilution and Reaction Buffer (Solution B) using ***a 1:10 dilution to make a 10 µg/ml stock***. Add the proper amounts of diluted 10 µg/ml rhMMP-13 (2-8 µl: 20-80 ng/well) to the wells. Add Solution B to wells to adjust the final volume to 90 µl/well.

2. **Add Proteinase Inhibitor**: Dissolve one vial of proteinase inhibitor in 1 ml of Solution B. Add 10 µl of proteinase inhibitor into all wells to neutralize non-collagenolytic proteinases in solution.

3. **Add Inhibitor Test Samples**: Add 1-50 µl of inhibitor test samples to the appropriate activated wells.

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4. **Add Solution B**: Add appropriate amounts of Sample Dilution and Reaction Buffer (Solution B) to the wells to adjust the final volume to 150 µl/well.

5. **Incubation**: Incubate rhMMP-13 and inhibitor test samples for 5 minutes to 60 minutes at room temperature.

   NOTE: The incubation time must be optimized depending on affinity or concentration of the inhibitor against rhMMP-13.

6. **Add Substrate**: Prepare substrate solution (20 µM) by diluting the 100X Substrate 1:100 with Substrate Dilution Buffer (Solution A). Add 100 µl (2 nmole) of diluted substrate solution to each well. Incubate at room temperature (25°C) for approximately 30 minutes. Incubation time depends on the amount of rhMMP-13 used.

7. **Add Stop Solution**: Stop the collagenase reaction by adding 10 µl of Stop Solution to each well.

8. **Read Plate**: Determine the fluorescence intensity (FI) at λEm = 450 nm and λEx = 365 nm or at λEm = 440 nm and λEx = 340 nm.

CALCULATING INHIBITION ACTIVITY

Calculate the inhibition % of test samples. Choose the standard point that uses the same amount of rhMMP-13 as is used with the sample. For example, in the Inhibition Assay Sheet, 40 ng/tube (4 µl of 10 µg/ml) is used for both Standard 2 and the inhibitor assays so the fluorescence intensity (FI) values of Standard 2 and samples must be used to calculate inhibition activity.

Inhibition % of test samples is calculated by comparing $FI_{Test\ Sample}$ to $FI_{Standard}$ as follows:

Subtract the blank FI value from the FI values of standards and test samples to obtain corrected FI values of standards ($FI_{Standard}$) and test samples ($FI_{Test\ Sample}$).

$FI_{Reference}$ = FI (Reference) - FI (Blank)

$FI_{Test\ Sample}$ = FI (Test Sample) - FI (Blank)

Inhibition % = $(1 - FI_{Reference} / FI_{Test\ Sample}) \times 100$

NOTE: If a different amount of rhMMP-13 is used, the amount must be within the linear range of the references for accurate results.

React 1 µg of rhMMP-13 (10 µl of undiluted rhMMP-13, 100 µg/ml) with 100 µl of substrate solution (20 µM), which contains 2 nmole of substrate at 25°C for 30 minutes. The fluorescence intensity (FI) obtained will reflect 100% of substrate (2 nmole) digested. By comparing the FI obtained for individual test samples to the FI of 2 nmole 100% digested substrate (Figure 2), the amounts of substrate (nmole) digested can be calculated.

Example: If the FI of 100% digested substrate was 10495, whereas FI observed in test sample 1 and test sample 2 were 1062 and 3845 respectively, actual amounts of substrate digested by samples are:

1062/10495 x 2 nmole = 0.202 nmole

3845/10495 x 2 nmole = 0.432 nmole

If the protein concentration in sample solution is known, divide the amount of substrate digested by the amount of protein in sample (µg or ng) to obtain specific activity of rhMMP-13 as nmole of substrate per µg or ng of protein.

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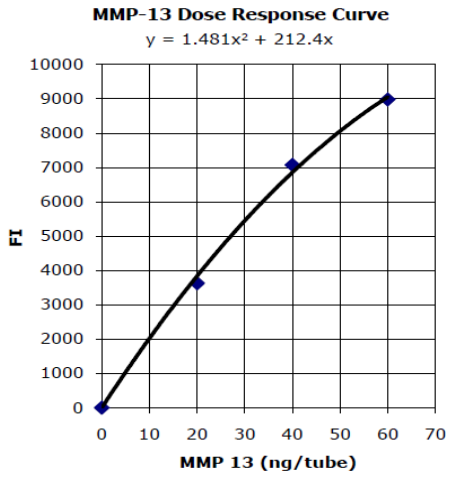

Figure 1- Dose Response Curve of rhMMP-13. rhMMP-13 (0, 20, 40, and 60 ng) was reacted with the fluorogenic peptide substrate (2 nmole) at 25°C for 30 minutes.

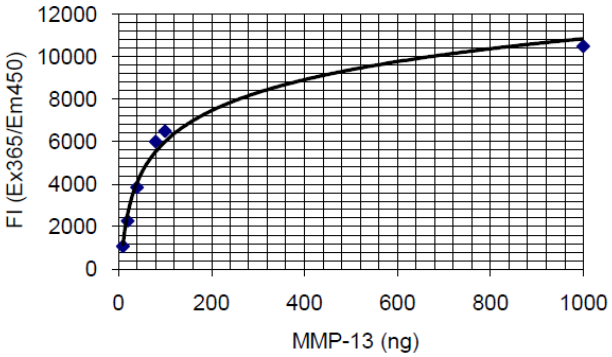

Figure 2 - Maximum Digestion of Fluorogenic Peptide Substrate by rhMMP-13. Fluorogenic peptide substrate (2 nmole) was completely digested by the reaction with 1000 ng of rhMMP-13 at 25°C for 30 minutes. This FI value shows 2 nmole of substrate and can be used to calculate actual amounts of substrate digested by the enzyme.

TROUBLESHOOTING

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

REFERENCES

1. M. P. Vincenti, Cloning of the gene for interstitial collagenase-3 (matrix metalloproteinase-13) from rabbit synovial fibroblasts: differential expression with collagenase-1 (matrix metalloproteinase-1). *J. Biochem*. **331(Pt. 1)**: 341-346 (1998).
2. P. G. Mitchel, e*t. al*, Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. *J. Clin. Invest.* **97(3)**: 761-768 (1996).
3. A. S. Fosang, Degradation of cartilage aggrecan by collagenase-3 (MMP-13). *FEBS Lett*. **380(1-2)**: 17-20 (1996).

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MMP-13 INHIBITOR ASSAY SHEET

This assay sheet is provided as a guide. Researchers will need to optimize the assay for their individual needs.

A) Preparing rhMMP-13

	Reference Curve					Inhibitor Assay		
	Blank	Reference 1	Reference 2	Reference 3	Reference 4	Inhibitor 1	Inhibitor 2	Inhibitor 3
Step 1 Reference rhMMP-13 Diluted 1:10 (10 µg/ml) (µl)	0	2	4	6	8	4	4	4
Step 2 Solution B (µl)	90	88	86	84	82	86	86	86
Step 3 Proteinase Inhibitor (µl)	10	10	10	10	10	10	10	10
Total Volume (µl)	100	100	100	100	100	100	100	100

B) Collagenase Activity Assay

	Reference Curve					Inhibitor Assay		
	Blank	Reference 1	Reference 2	Reference 3	Reference 4	Inhibitor 1	Inhibitor 2	Inhibitor 3
Step 1 Activated rhMMP-13 from Part A (µl)	100	100	100	100	100	100	100	100
Step 2 Inhibitor Test Sample (µl)	0	0	0	0	0	12.5	25	50
Step 3 Solution B (µl)	50	50	50	50	50	37.5	25	0
Incubate at 25°C (room temperature) for 5 - 60 minutes. NOTE: Incubation time depends on the inhibitor samples								
Step 4 Substrate Solution (µl)	100	100	100	100	100	100	100	100
Incubate at 25°C (room temperature) for 30 minutes. NOTE: Incubation time depends on the amount of rhMMP-13 used.								
Step 5 Stop Solution (µl)	10	10	10	10	10	10	10	10
Total Volume (µl)	260	260	260	260	260	260	260	260
Read fluorescence intensity (FI) at λEm = 450 nm and λEx = 365 nm or at λEm = 440 nm and λEx = 340 nm.								

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