Bacterial Collagenase Assay Kit

Catalog # 3014

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INTRODUCTION

Collagenase produced by *Clostridium histolyticum*, isolated by Mandl *et al.* in 1953 [1], has been widely used in research and clinical fields for isolating cells and digesting connective tissues [2]. Importantly, bacterial collagenases differ from mammalian collagenases in their substrate specificity, as bacterial collagenases cleave the native collagen molecule into multiple fragments, whereas mammalian collagenases cleave the collagen molecule at a single site into two fragments.

Clostridium species infection causes severe tissue necrosis leading to gas gangrene [3]. At the infection site, collagenases from *Clostridium* species facilitate extensive destruction of the extracellular matrix without regulation because of the absence of bacterial collagenase inhibitors in animal and human sera, whereas mammalian collagenase activity is strictly regulated by mammalian serum inhibitors [4-6].

Chondrex, Inc. provides a rapid bacterial collagenase assay kit using soluble FITC-labeled type I collagen instead of radio-labeled collagen [7] as a substrate. This kit can be used not only for assaying collagenase activity, but also for inhibitor assays. Please refer to page 2 for the Collagenase Activity Assay protocol or page 4 for the Collagenase Inhibitor Assay protocol.

Note: The reference collagenase is provided in this kit as a positive control, not as a standard. Collagenase activity in samples should be determined based on the amounts of collagen substrate digested.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Reference *Clostridium Histolyticum* Collagenase (30141)	2 vials	100 units lyophilized	-20°C
Solution A - FITC-Collagen Dilution Buffer (30041)	1 bottle	10 ml	-20°C
Solution B - Sample Dilution and Reaction Buffer (30042)	1 bottle	50 ml	-20°C
2X FITC-Labeled Bovine Type I Collagen (4001)	1 bottle	10 ml, 1 mg/ml in 0.01M acetic acid	-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	-20°C
Extraction Buffer (30048)	2 bottles	25 ml	-20°C
ELISA Plate (Black)	1 plate	96-well	-20°C

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ASSAY PROCEDURE

A. Collagenase Activity Assay

1. Prepare 1.5 ml amber colored microcentrifuge tubes as shown in the collagenase assay sheet on page 3.

2. Dissolve one vial of Reference collagenase in 1 ml of Solution B. Partially used Reference collagenase stock solution can be kept at - 20°C for future assays.

3. Add the proper amounts of Reference collagenase, test samples diluted with Solution B, and Solution B as shown in the collagenase assay sheet.

   Note 1: **Because bacterial collagenase exists as an active form, collagenase activation is not necessary.**

   Note 2: The final volume should be 190 μl in all tubes, except the Buffer tube.

4. Dissolve one vial of Proteinase Inhibitor in 1 ml of Solution B. Add 10 μl of Proteinase Inhibitor to all tubes to inhibit the non-collagenolytic proteinases in the test samples.

5. Prepare the substrate solution by mixing an equal volume of 2X FITC-collagen and cold Solution A (4°C) in an amber colored tube or bottle as FITC is light sensitive.

   Note: 200 μl of this 1X FITC-collagen solution is required for all tubes, except the Buffer tube.

6. Add 200 μl of 1X FITC-collagen solution to all tubes except the Buffer tube. Mix well and incubate at 35°C for 10-120 minutes.

   Note 1: Incubate the reference collagenase tubes and 100% control tube for 60 minutes at 35°C.

   Note 2: The incubation time of test samples will vary depending on the collagenase activity. Do not incubate more than 120 minutes, as background levels will increase. Background refers to the degradation of collagen due to extended exposure to high temperatures.

7. Stop the collagenase reaction by adding 10 μl of Stop Solution to each tube and mixing well.

8. Cool samples to room temperature. Add 400 μl of Extraction Buffer (room temperature) to each tube. Mix vigorously and centrifuge at 10,000 rpm for 10 minutes.

9. Transfer 200 μl of each supernatant into a black 96-well plate and determine the fluorescence intensity (FI) at λem = 520 nm and λex = 490 nm.

CALCULATION OF COLLAGENASE ACTIVITY

One unit of collagenolytic activity is defined as the cleavage of 1 μg of collagen per minute. Because 100 μg of collagen is used as a substrate per test in this assay kit, collagenolytic activity is calculated by the following equation:

$$\text{Collagenase Activity (units/ml)} = \frac{(FI_{sample} - FI_{blank}) \times 100\ \mu g}{(FI_{control} - FI_{blank}) \times \text{Reaction Time (minutes)} \times \text{Sample Volume (ml)}}$$

FI_{blank} = FI in blank
$FI_{control}$ = FI in 100% control
FI_{sample} = FI in test samples

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Collagenase Assay Sheet

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

	Buffer	Control (100%)	Blank	Ref 1	Ref 2	Ref 3	Ref 4	Test Sample
Step 1 Add Reference Collagenase (µl)	0	50	0	2.5	5	7.5	10	0
Step 2 Add Test Sample (µl)	0	0	0	0	0	0	0	1-100
Step 3 Add Solution B (µl)	390	140	190	187.5	185	182.5	180	189-90
Step 4 Add Proteinase Inhibitor (µl)	10	10	10	10	10	10	10	10
Total Enzyme Solution (µl)	400	200	200	200	200	200	200	200
Step 5 Add 1X FITC-Collagen (µl)	0	200	200	200	200	200	200	200
React at 35°C for 10-120 minutes.								
Step 6 Add Stop Solution (µl)	10	10	10	10	10	10	10	10
Step 7 Add Extraction Buffer (µl)	400	400	400	400	400	400	400	400
Mix well and centrifuge at 10,000 rpm for 10 minutes.								
Transfer 200 µl of supernatant into a 96-well flat bottom black plate.								
Step 8 Determine FI at Em 520/Ex 490	FI_{blank}	$FI_{control}$	$FI_{(0)}$	$FI_{(2.5)}$	$FI_{(5)}$	$FI_{(7.5)}$	$FI_{(10)}$	$FI_{(sample)}$
Calculate collagenase activity by comparing FI.								

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ASSAY PROCEDURE

B. Collagenase Inhibitor Assay

1. Prepare 1.5 ml amber colored microcentrifuge tubes as shown in the collagenase inhibitor assay sheet on page 5.

2. Dissolve one vial of Reference collagenase in 1 ml of Solution B. Partially used Reference collagenase stock solution can be kept at -20°C for the next assay.

3. Add the proper amounts of Reference collagenase, test samples diluted with Solution B, and Solution B as shown in the collagenase inhibitor assay sheet.

   Note: The final volume should be 190 μl in all tubes.

4. Dissolve one vial of Proteinase Inhibitor in 1 ml of Solution B. Add 10 μl of Proteinase Inhibitor to all tubes to inhibit the non-collagenolytic proteinases in the test samples.

5. Prepare the substrate solution by mixing an equal volume of 2X FITC-collagen and cold Solution A (4°C) in an amber colored tube or bottle as FITC is light sensitive.

   Note: 200 μl of this 1X FITC-collagen solution is required for all tubes.

6. Add 200 μl of 1X FITC-collagen solution to all tubes except the Buffer tube. Mix well and react at 35°C for 60 minutes.

7. Stop the collagenase reaction by adding 10 μl of Stop Solution to each tube, and mix well.

8. Cool samples to room temperature. Add 400 μl of Extraction Buffer (room temperature) to each tube. Mix vigorously and centrifuge at 10,000 rpm for 10 minutes.

9. Carefully transfer 200 μl of each supernatant into a black 96-well plate and determine the fluorescence intensity (FI) at λem = 520 nm and λex = 490 nm.

Note: Supernatants contaminated with pellets in the 96-well plate will lead to high FI values, resulting in overestimated assay results

CALCULATION OF COLLAGENASE ACTIVITY

One unit of collagenolytic activity is defined as the cleavage of 1 μg of collagen per minute. Because 100 μg of collagen is used as a substrate per test in this assay kit, collagenolytic activity in the reference and test sample tubes are calculated by the following equation:

$$\text{Collagenase Activity (units/ml)} = \frac{(FI_{\text{sample OR reference}} - FI_{\text{blank}}) \times 100\ \mu g}{(FI_{\text{control}} - FI_{\text{blank}}) \times 60\ \text{(minutes)} \times 0.1\ \text{(ml)}}$$

FI_{blank} =FI in blank
$FI_{control}$ = FI in 100% control
FI_{sample} = FI in test samples

Note: Supernatants contaminated with pellets in the 96-well plate will lead to high FI values, resulting in overestimated assay results

$$\text{\% INHIBITION} = \frac{\text{Collagenase activity (sample)}}{\text{Collagenase activity (reference)}} \times 100$$

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Collagenase Inhibitor Assay Sheet

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

	100% Control	Blank	Reference	Test Sample
Step 1 Add Reference Collagenase (µl)	50	0	10	10
Step 2 Add Test Sample (µl)	0	0	0	1-100
Step 3 Add Solution B (µl)	140	190	180	179-80
Step 4 Add Proteinase Inhibitor (µl)	10	10	10	10
Total Enzyme Solution (µl)	200	200	200	200
Step 5 Add 1X FITC-Collagen (µl)	200	200	200	200
React at 35°C for 60 minutes.				
Step 6 Add Stop Solution (µl)	10	10	10	10
Step 7 Add Extraction Buffer (µl)	400	400	400	400
Mix well and centrifuge at 10,000 rpm for 10 minutes.				
Transfer 200 µl of supernatant into a 96-well flat bottom black plate.				
Step 8 Determine FI at Em 520/Ex 490	$FI_{control}$	FI_{blank}	$FI_{reference}$	FI_{sample}
Calculate collagenase activity by comparing FI.				

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