Version 8, Last updated 23 November 2023

ab196999 Collagenase Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of collagenase activity in bacterial extract or purified protein.

View kit datasheet: www.abcam.com/ab196999
(use www.abcam.cn/ab196999 for China, or www.abcam.co.jp/ab196999 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Collagenase Activity Assay Kit (colorimetric) (ab196999) provides a quick and easy way to determine activity of collagenase. This assay measures collagenase activity using a synthetic peptide (FALGPA) that mimics the structure of collagen. It is suitable for measuring activity of bacterial collagenases such as from *Clostridium histolyticum* type I-XI. In addition, this product can also be used to screen/characterize collagenase inhibitors. The limit of detection for this assay is 0.02 mU collagenase.

Collagenase (EC 3.4.24.3) is an enzyme in the matrix metalloproteinase family that breaks down collagen, assisting in degradation of the extracellular matrix, which is a key step in the pathogenesis of bacteria. Collagen is an abundant structural protein present in the connective tissue of animals. Collagenase has been used clinically for the treatment of Dupuytren's contracture, an affliction characterized by a thickening of connective tissue.

2. Protocol Summary

Sample preparation

Add reaction mix

Measure absorbance (OD345 nm) in a kinetic mode
for 5-10 minutes at 37°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Collagenase Assay Buffer	20 mL	-20°C	4°C/-20°C
Collagenase	1 mL	-20°C	-20°C
Collagenase Substrate I/Collagenase Substrate (FALGPA)	4 mL	-20°C	-20°C
Inhibitor (1,10-Phenanthroline)	50 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 345 nm
- Double distilled water (ddH_2O)
- PBS
- HBSS (Hank's Balanced Salt Solution)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Collagenase Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C or 4°C.

9.2 Collagenase positive control:

Ready to use as supplied. Thaw on ice. Aliquot Collagenase so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

9.3 Collagenase Substrate I/Collagenase Substrate (FALGPA):

Ready to use as supplied. Thaw on ice. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

9.4 Inhibitor (1,10-Phenanthroline) (1 M):

Ready to use as supplied. Thaw on ice. Aliquot inhibitor so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

10. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

10.1 Purified collagenase:

Dissolve test collagenase in cold ddH_2O or HBSS.

Suggested range for collagenase testing: 0.02-10 mU

10.2 Bacterial extracts:

10.2.1 Lyse bacterial cells in cold PBS.

10.2.2 Centrifuge lysates 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.

10.2.3 Collect supernatant and transfer to a new tube.

10.2.4 Keep on ice.

10.3 Inhibitor Screening compounds:

10.3.1 Dissolve test inhibitors to a 100X solution into appropriate solvent.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

11. Assay Procedure – Collagenase Activity

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- Positive control and inhibitor control are provided to ensure the assay is working correctly. They shouldn't be used as standard or to extrapolate enzyme activity from the sample.

11.1 Reaction wells set up:

- Reagent background well = 100 µL/well Assay Buffer
- Sample wells = 2-10 µL samples (adjust volume to 100 µL/well with Assay Buffer).
- Positive control well = 10 µL Collagenase + 90 µL Assay Buffer.
- Inhibitor control well = 10 µL Collagenase + 2 µL Inhibitor + 88 µL Assay Buffer

The table below shows the reaction set up:

Component	Sample well (µL)	Positive control (µL)	Inhibitor well (µL)	Background well (µL)
Sample test	2-10	-	-	-
Collagenase	-	10	10	-
1,10-Phenanthroline	-	-	2	-
Assay Buffer	Up to 100	90	88	100

11.2 Collagenase Reaction mix:

11.2.1 Prepare 100 µL of Collagenase Reaction Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)
Collagenase Assay Buffer	60
Collagenase Substrate I/Collagenase Substrate	40

11.2.2 Add 100 µL of Reaction Mix into each well. Mix well and proceed immediately to measure activity.

11.3 Measurement:

11.3.1 Measure output immediately at OD 345 nm on a microplate reader in kinetic mode for at least 5-15 minutes at 37°C protected from light.

Δ Note: Low activity samples can be measured for 1-3 hours. High activity samples will consume substrate within 3 minutes. Dilute enzyme and measure again if necessary.

Δ Note: Incubation time depends on the collagenase activity in the samples. We recommend measuring OD in a kinetic mode and choosing two time points (T1 and T2) to calculate the collagenase activity of the samples.

12. Assay Procedure – Inhibitor Screening

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Positive control and inhibitor control are provided to ensure the assay is working correctly. They shouldn't be used as standard or to extrapolate enzyme activity from the sample.

12.1 Reaction wells set up:

- Reagent background well = 100 µL/well Assay Buffer
- Inhibitor test sample wells = 10 µL Collagenase + 2 µL test inhibitor + 88 µL Assay Buffer.
- Positive control well = 10 µL Collagenase + 90 µL Assay Buffer.
- Inhibitor control well = 10 µL Collagenase + 2 µL Inhibitor + 88 µL Assay Buffer
- Solvent control wells = 10 µL Collagenase + 2 µL solvent + 88 µL Assay Buffer

The table below shows the reaction set up:

Component	Sample well (µL)	Posit contr (µL)	Inhibitor contr (µL)	Solvent (µL)	Bckg well (µL)
Inhibitor test	2	-	-	-	-
Collagenase	10	10	10	10	-
Inhibitor control	-	-	2		-
Solvent	-	-	-	2	-
Assay Buffer	88	90	88		100

12.2 Collagenase Reaction mix:

12.2.1 Prepare 100 µL of Collagenase Reaction Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)
Collagenase Assay Buffer	60
Collagenase Substrate I/Collagenase Substrate	40

12.2.2 Add 100 µL of Reaction Mix into each well. Mix well and proceed immediately to measure activity.

12.3 Measurement:

12.3.1 Measure output immediately at OD 345 nm on a microplate reader in kinetic mode for at least 5-15 minutes at 37°C protected from light.

Δ Note: Low activity samples can be measured for 1-3 hours. High activity samples will consume substrate within 3 minutes. Dilute enzyme and measure again if necessary.

Δ Note: Incubation time depends on the collagenase activity in the samples. We recommend measuring OD in a kinetic mode and choosing two time points (T1 and T2) to calculate the collagenase activity of the samples.

13. Calculations

– Use only the linear rate for calculation

13.1 Measurement of collagenase activity in the sample:

13.1.1 For all reaction wells (including background control samples), choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding OD values at those points (OD1 and OD2).

Δ Note: readings should have at least two reading in between and be more than 1 minute apart.

13.1.2 Calculate ΔOD for sample as follows:

ΔOD345nm = A1 – A2

13.1.3 Determine the background corrected change in fluorescence intensity for each well of sample by subtracting the ΔOD value of the background control well.

13.1.4 Collagenase activity (U/mL) in the test samples, positive control and inhibitor control is calculated as:

$$Collagenase\ Activity = \frac{\left(\frac{\Delta ODc}{\Delta T}\right) x\ 0.2\ x\ D}{0.53\ x\ V}$$

Where:

ΔODc = ΔOD reading from sample at T2 and T1, corrected for background (Step 13.1.3).

ΔT = linear phase reaction time T2 – T1 (minutes).

0.2 = reaction volume (mL).

D = sample dilution factor.

0.53 = millimolar extinction coefficient of FALGPA

V =sample volume added into the reaction well (mL).

Collagenase activity can also be expressed as U/mg of total protein in the sample.

13.2 Inhibitor compound screening:

13.2.1 For all reaction wells (including background control samples), choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding OD values at those points (OD1 and OD2).

Δ Note: readings should have at least two reading in between and be more than 1 minute apart.

13.2.2 Calculate ΔOD for each inhibitor compound tests, inhibitor control and enzyme control as follows:

ΔOD345nm = A1 – A2

13.2.3 Determine the background corrected change in fluorescence intensity for each well of sample by subtracting the ΔOD value of the background control well.

13.2.4 Collagenase activity (U/mL) is calculated as:

$$Collagenase\ Activity = \frac{\left(\frac{\Delta ODc}{\Delta T}\right) x\ 0.2\ x\ D}{0.53\ x\ V}$$

Where:

ΔODc = ΔOD reading from sample at T2 and T1, corrected for background (Step 12.1.3).

ΔT = linear phase reaction time T2 – T1 (minutes).

0.2 = reaction volume (mL).

D = sample dilution factor.

0.53 = millimolar extinction coefficient of FALGPA

V =sample volume added into the reaction well (mL).

13.2.5 For inhibitor screening, calculate percentage of inhibition using the following calculation:

$$\%\ Inhibition = \frac{Activity_{(Enzyme)} - Activity_{(Inhibitor)}}{Activity_{(Enzyme)}} \times 100$$

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

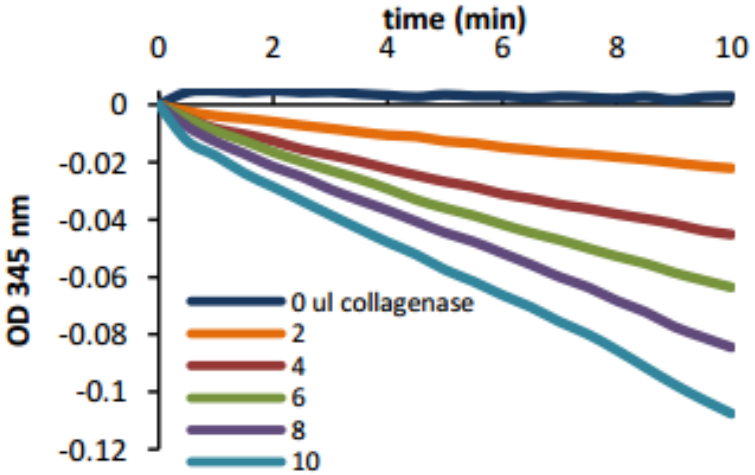

Figure 1. Typical collagenase activity curve from positive control, obtained over a 10 minute-reading period.

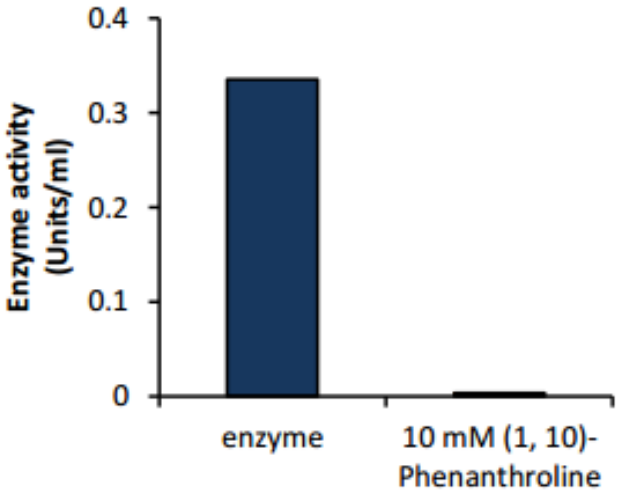

Figure 2. Example of enzyme activity of provided positive and inhibitor control.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate as indicated in table below:

COLLAGENASE ACTIVITY

Component	Sample well (µL)	Positive control (µL)	Inhibitor well (µL)	Background well (µL)
Sample test	2-10	-	-	-
Collagenase	-	10	10	-
1,10-Phenanthroline	-	-	2	-
Assay Buffer	Up to 100	90	88	100

COLLAGENASE INHIBITOR SCREENING

Component	Sample well (µL)	Posit contr (µL)	Inhibitor contr (µL)	Solvent (µL)	Bckg well (µL)
Inhibitor test	2	-	-	-	-
Collagenase	-	10	10	10	-
Inhibitor control	-	-	2		-
Solvent	-	-	-	2	-
Assay Buffer	98	90	88		100

- Prepare a master mix for Reaction Mix: 60 µL Collagenase Assay Buffer + 40 µL Collagenase Substrate I/Collagenase Substrate (100 µL/reaction)
- Add 100 µL Reaction to sample wells.
- Measure plate immediately at OD 345 nm on a microplate reader in a kinetic mode at 37°C for 5-15 minutes protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQs

Q. I have used your product twice but the readings I get from the collagenase positive control are different. What is the problem?

A. The collagenase provided in the kit as positive control is an active recombinant enzyme, and its activity might be different with each production batch. Regardless of its activity, we provide the same amount so that you don't have change the procedure. However, it is very likely that the enzyme activity will vary from batch to batch.

Regardless of the specific activity of each collagenase batch, you should see high absorbance on the positive control wells and very low absorbance on the inhibitor control wells.

Q. What can't I use your assay to measure mammalian collagenase activity?

A. Bacterial and vertebrate collagenases have different substrate specificity requirements. The substrate used in this assay, FALGPA, is specific for bacterial collagenase and won't be efficiently converted by vertebrate (and therefore, mammalian) collagenases.

18. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)