

QuickZyme

Total Collagen

Assay

Updated Version July 2011

This package insert must be read in its entirety before using this product.



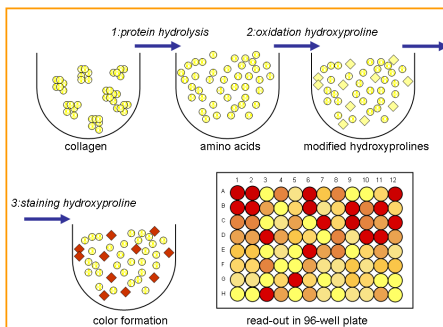
Introduction

Collagen is one of the main components of extracellular matrix. Dysregulation in collagen production results in pathologies such as fibrosis (too much collagen), or osteoarthritis (too little collagen). Therefore measurement of collagen production is important in many disease related studies.

The QuickZyme total collagen assay is based on the detection of hydroxyproline. Hydroxyproline is a non-proteinogenic amino acid, which in mammals occurs in elastin and collagen. Its presence is mainly limited to the triple helix of collagen, where its presence increases the triple helix stability. Hydroxyproline is formed post-translationally from specific proline residues by action of the enzyme prolylhydroxylase. Hydroxyproline in tissue hydrolysates can be used as a direct measure of the amount of collagen present.

The measurement of collagen is started by complete hydrolysis of tissue samples in 6M HCl at 95°C. In the hydrolysate hydroxyproline residues are quantified using a modification of the method described by Prockop and Udenfriend (Anal. Biochem., 1960, 1: 228-239). The assay measures the total amount of hydroxyproline present in the sample, which represents all the types of collagen present in the sample without discriminating between the types of collagen and between procollagen, mature collagen and collagen degradation products. The assay is simple and results in a chromogen with an absorbance maximum at 570 nm. The assay is developed in such a way that it doesn't need the drying step following acid hydrolysis for which often special equipment is needed.

Assay principle



What's in the box?

- 96 special screw-capped tubes needed for sample hydrolysis
- 2 adhesive plate seals
- Assay buffer
- Detection reagent A
- Detection reagent B
- Collagen standard (3000 µg/ml) in 0.02M acetic acid
- 96-well assay plate
- Assay protocol booklet

Other materials required

The following materials and equipment are required but not supplied:

- 12M and 6M HCl for sample hydrolysis
- 4 M HCl for sample dilution
- Single and/or multichannel pipettes
- Eppendorf centrifuge
- Incubator (or thermoblock or oven) for heating at 95°C
- Incubator (or oven) for heating at 60°C
- Microplate reader capable of measuring at a wavelength between 540 and 580 nm, 570 nm preferred.
- Microplate shaker

Storage conditions

Unopened kit:

Store at room temperature (RT) in the dark. Do not use kit components past kit expiration date.

Opened kit / reconstituted reagents:

The opened collagen standard should be stored at reagents should be stored at 4°C. The other opened reagents should be stored light protected at RT and are stable for at least 2 months. The reconstituted detection reagent (A+B) should be stored light protected at RT and is stable for at least 1 month upon reconstitution.

Precaution

The kit contains n-propanol, perchloric acid, acetic acid and DMSO. See for relevant MSDS:

www.quickzyme.com/products/total-collagen-assay.

Wear eye, hand, face, and clothing protection during hydrolysis of the samples and when using the kit. Perform the assay in a fume hood.

Critical parameters

- Hydrolysis takes place at 95°C for 20 hrs. *The screw-capped tubes should be tightly closed by hand. If tubes are not tightly closed, hydrolysis solution will evaporate and the sample will be lost.*
- The incubation time for color development at 60°C during the last step of the assay is 1hr. This is based on incubation in an oven. When incubation is performed in a plate incubator (with tight contact between incubator and plate) a reduced incubation time (20-30 min) is sufficient.
- When 35 µl of the (diluted) hydrolyzed sample is added to the assay buffer, a cloudy appearance can develop, that will disappear within a minute and does not influence the assay.
- At low temperature the assay buffer may contain some crystals. These can be dissolved by warming.
- If the hydroxyproline level in the hydrolyzed samples is too low for detection, samples can be concentrated by drying the hydrolyzed samples and redissolve them in a smaller volume of 4M HCl.

Buffer / reagent preparation

- Assay buffer is ready for use
- For preparation of the detection reagent mix 2 volumes of detection reagent A with 3 volumes detection reagent B.

Sample preparation (1)

- Conditioned culture medium / cell extracts

Conditioned media of cell cultures or cellular extracts (50 – 250 µl) are transferred to the screw-capped tubes and are diluted 1:1 (v/v) with **12M** HCl (final concentration **6M** HCl). A minimum of 50 µl sample and 50 µl 12 M HCl is advised. Close tubes tightly before placing in oven. See also critical parameters above.

- Tissues

A. Tissue homogenates

Tissue homogenates are treated similarly as described above for conditioned culture medium and cell extracts.

B. Tissue samples

Tissue samples (either wet or dried) are weighed and transferred to the screw-capped tubes. The amount of tissue needed is highly dependent on the collagen level in the tissue. As an indication, dependent on the type and amount of tissue, add **6M** HCl to obtain 50-300 mg tissue/ml. A minimum volume of 100 µl is advised. Close tubes tightly (see Critical parameters and Sample preparation (2)) before placing in oven.

Sample preparation (2)

Tubes must be closed very tightly (rubber ring should become well visible from above) and incubated for 20 hrs at 95°C in an oven or thermoblock (do not incubate at higher temperature). After incubation the tubes are cooled to room temperature. Do not open tubes until they have reached room temperature. Tubes are centrifuged for 10 min at 13,000 x g in an Eppendorf centrifuge. *Hydrolyzed samples will need to be diluted (dependent on the sample, but at least twofold). All dilutions should be performed using **4M** HCl.* 35 µl of the (diluted) hydrolyzed sample is used for analysis in the assay.

Standard preparation

The collagen standard is provided as a stock of 3000 µg/ml in 0.02M acetic acid.

For a standard line 90 µl of this standard is transferred to a screw-capped tube and mixed with an equal volume (90 µl) of **12M** HCl (final concentration 1500 µg/ml in **6M** HCl). The tube is closed very tightly (rubber ring should become well visible) and incubated for 20 hrs at 95°C in an oven or thermoblock. After incubation the tube is cooled to room temperature and centrifuged for 10 min in an Eppendorf centrifuge at 13,000 x g.

Label 8 Eppendorf tubes as S1-S8. S1 to S7 are dilutions of the hydrolyzed stock and S8 is a blank. The standard dilutions are made with **4M** HCl according to the scheme below.

This results in a standard line as follows: 300 µg/ml (S1); 200 µg/ml (S2); 100 µg/ml (S3); 50 µg/ml (S4); 25 µg/ml (S5); 12.5 µg/ml (S6); 6.25 µg/ml (S7); 0 µg/ml (S8). Mix all the standards well upon dilution.

35 µl of each standard is used for analysis in the assay.

Standard label	Sample from	4M HCl	Conc (µg/ml)	Pipetting scheme for the preparation of the samples for the collagen standard line
S1	50 µl hydr. stock	200 µl	300	
S2	120 µl S1	60 µl	200	Assay procedure
S3	90 µl S2	90 µl	100	<i>It is recommended that all samples and standards are assayed in duplicate</i>
S4	90 µl S3	90 µl	50	1. Prepare the samples as described in 'sample preparation'.
S5	90 µl S4	90 µl	25	2. Prepare the collagen standard as described in 'standard preparation'.
S6	90 µl S5	90 µl	12.5	3. Pipette 35µl standard into appropriate wells of the assay microplate.
S7	90 µl S6	90 µl	6.25	4. Pipette 35 µl of the (diluted) hydrolyzed unknown samples into the appropriate wells. Dilutions should be performed with 4M HCl.
S8	0 µl	90 µl	0	

5. Add 75 µl assay buffer to each well.
6. Cover the plate with an enclosed adhesive plate seal and incubate 20 minutes at room temperature, while shaking the plate.
7. Prepare a volume of detection reagent sufficient for the number of wells to be tested (75 µl/well) by mixing detection reagents A and B 2:3 (resp 30 µl +45 µl/well).
8. Add 75 µl detection reagent to each well.
9. Cover the plate with an enclosed adhesive plate seal.
10. Mix well by shaking the plate. Incubate 60 minutes at 60°C in an oven.
11. Cool the plate on ice to room temperature.
12. Clean the bottom of the plate and read the plate at 570 nm (540-580 nm acceptable) and perform data analysis.

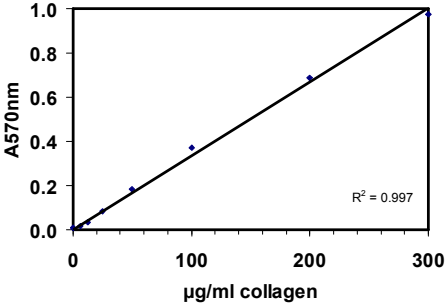
Data analysis

Several options are available for the calculation of the collagen concentration in the assay samples. It is recommended that the data are handled by a software package utilizing a regression curve fitting program. If not available, the collagen concentration can be calculated manually as follows.

- Average the duplicate readings for each standard or sample and subtract the average blank from all readings.
- Create a standard curve by plotting the mean A_{570} (minus blank) of each standard on the y-axis against the collagen content on the x-axis (0- 3.13 – 6.25 – 12.5 – 25 - 50 – 100 – 200 - 300 µg collagen/ml hydrolysate). Draw a best-fit linearized curve through the points on the graph. Use this standard curve to convert the A_{570} values of the test samples to µg/ml collagen. This gives the collagen concentration in the hydrolyzed sample. If after hydrolysis a dilution step is included, the concentration should be multiplied with the dilution factor to give the collagen concentration in the hydrolyzed sample. Depending on the sample preparation the amount of collagen in the original samples can be calculated.

Typical data

The shown data curve is provided for demonstration only. The exact A_{570} values can vary slightly per experiment.



A typical collagen standard curve in the range of 6 - 300 µg/ml collagen).

Other related products

- Soluble collagen assay
- Hydroxyproline assay
- Human MMP-9 activity assay
- Mouse MMP-9 activity assay
- Human MMP-2 activity assay
- Mouse MMP-2 activity assay
- Human Granzyme B activity assay reagent set
- Human BACE-1 activity assay

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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