

QuickZyme

Hydroxyproline

Assay

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



Introduction

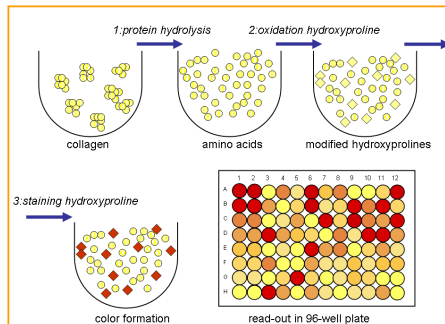
Dysregulation in collagen metabolism may result in pathologies such as fibrosis (too much collagen), or osteoarthritis (too little collagen). Therefore measurement of collagen production is important in many disease related studies.

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 it leads to increased 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 QuickZyme hydroxyproline assay is 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. If this hydroxyproline is obtained upon hydrolysis of collagen, it 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 to measure hydroxyproline in acid tissue and/or protein hydrolysates in such a way that it doesn't need the drying step following acid hydrolysis of collagen for which often special equipment is needed.

Assay principle



What's in the box?

- 2 adhesive plate seals
- Assay buffer
- Detection reagent A
- Detection reagent B
- Hydroxyproline standard (3 mM) in sterile water
- 96-well assay plate
- Assay protocol booklet

Other materials required

The following materials and equipment are required but not supplied:

- 4 M HCl for sample and standard dilution
- MilliQ or comparable high quality water
- Single and/or multichannel pipettes
- Eppendorf centrifuge
- 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 in the dark. Do not use kit components past kit expiration date.

Opened kit / reconstituted reagents:

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

Precaution

The kit contains n-propanol, perchloric acid, and DMSO. See for relevant MSDS: www.quickzyme.com/products/hydroxyproline-assay.

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

Critical parameters

- The samples used in the assay should be present in a solution containing 4-6M HCl.
- 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 hydrolysate 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.

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

The QuickZyme hydroxyproline assay is developed to measure hydroxyproline in acid hydrolysates, e.g. from conditioned culture medium, cell extracts, tissue homogenates, wet or dry tissue samples. These samples should have been hydrolyzed in 6N HCl (final concentration) according to established procedures. After hydrolysis the tubes are cooled down to 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). Dilution should be performed using 4M HCl.

35 µl of the (diluted) hydrolysate is used for analysis in the assay.

Standard preparation

The hydroxyproline standard is provided as a stock solution of 3 mM in sterile water.

For a standard line 8 Eppendorf tubes are labeled as S1-S8. S1 to S7 are dilutions of the stock solution 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 μ M (S1); 200 μ M (S2); 150 μ M (S3); 100 μ M (S4); 50 μ M (S5); 25 μ M (S6); 12.5 μ M (S7); 0 μ M (S8). Mix all the standards well upon dilution.

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

Standard label	Sample from	4M HCl	Conc (μ M)
S1	30 μ l stock	270 μ l	300
S2	120 μ l S1	60 μ l	200
S3	45 μ l S1	45 μ l	150
S4	90 μ l S2	90 μ l	100
S5	90 μ l S4	90 μ l	50
S6	90 μ l S5	90 μ l	25
S7	90 μ l S6	90 μ l	12.5
S8	0 μ l	90 μ l	0

Pipetting scheme for the preparation of the samples for the collagen standard line

Assay procedure

It is recommended that all samples and standards are assayed in duplicate

1. Prepare the samples as described in 'sample preparation'
2. Prepare the hydroxyproline standard as described in 'standard preparation'
3. Pipette 35 μ l standard into appropriate wells of the assay microplate
4. Pipette 35 μ l of each (diluted) sample into the appropriate wells. Depending on the hydroxyproline content of the sample a dilution step might be required. Dilution should be performed in 4M HCl
5. Add 75 μ l 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 μ l/well) by mixing detection reagents A and B 2:3 (resp 30 μ l +45 μ l/well)
8. Add 75 μ l 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.

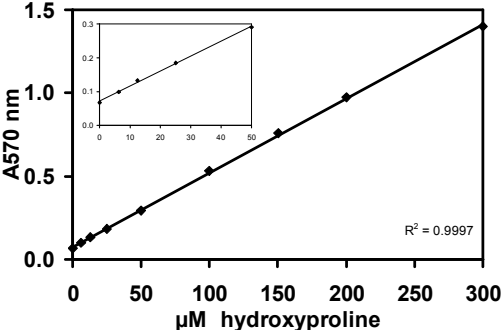
Data analysis

Several options are available for the calculation of the hydroxyproline 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 hydroxyproline 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 hydroxyproline content on the x-axis (0- 12.5 – 25 - 50 – 100 – 150 - 200 - 300 μ M hydroxyproline). 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 μ M hydroxyproline. This gives the hydroxyproline concentration in the hydrolysate. If after hydrolysis a dilution step is included, the concentration should be multiplied with the dilution factor to give the hydroxyproline concentration in the hydrolysate. Depending on the sample preparation the amount of hydroxyproline in the original samples can be calculated.

Typical data

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



A typical hydroxyproline standard curve in the range of 6 - 300 μM hydroxyproline.

Other related products

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

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

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