QuickZyme
Soluble Collagen Assay

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

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

QuickZyme Biosciences

Introduction

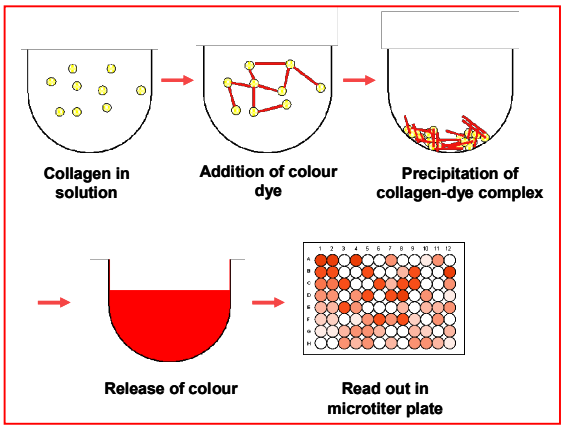
Collagen is one of the main components of extracellular matrix. Dysregulation in collagen production results in pathologies such as fibrosis (too much collagen), or osteoarthritis (too little collagen). Therefore measurement of collagen production is important in many disease related studies.

The QuickZyme collagen assay is based on binding of the dye Sirius Red to collagen. Following binding of the dye, the collagen-dye complex precipitates, resulting in a coloured pellet. This colour can be released in an alkaline solution.

The assay measures various types of fibrillar collagen. The most abundant collagen types are collagen type I (skin, bone, vessels, connective tissue), type II (cartilage) and type III (skin, bone, connective tissue).

The assay is specific for collagen. After heating at 50°C the signal is lost, since the assay does not recognize denatured collagen (gelatin).
The assay can be used to analyze soluble
collagen or collagen that is solubilized by acetic acid alone or in combination with pepsin. The assay is intended for collagen analysis in conditioned cell culture media and cellular extracts. The assay is not affected by serum up to at least 10%.

Assay principle



What's in the box?

- *Plate Set and balance plate set*, each containing:
  - *1 Assay microplate* - 96 well polypropylene V-shaped microplate. Depending on the number of samples the plate can be cut for partial use.
  - *1 Reading microplate* - 96 well clear polystyrene flat bottom microplate. This plate is used for the measurement of absorbance at 540 nm in a microplate reader; it can also be used as a holder for the assay plate during centrifugation.
  - 1 *adhesive plate seal*
- *Collagen standard solution* - Rat tail type 1 collagen, 714 µg/ml in 20 mM acetic acid. The solution can be used for multiple standard lines.
  Sarstedt tube 1.5 ml (containing 500 µl)
- *Dye solution* - Sterile solution containing Sirius Red dye.
  Plastic bottle 15 ml (containing 8 ml)

- *Dilution buffer* - Sterile buffer for blanks and standard / sample dilution.
  Plastic bottle 30 ml (containing 20 ml)
- *Wash buffer* - Buffer containing detergent (inflammable).
  120 ml bottle (containing 80 ml)
- *Detection solution* - Sterile alkaline buffer.
  30 ml bottle (containing 18 ml)

Precaution

The standard collagen solution in this kit is an acid solution. The dilution buffer contains 0.01% sodium azide. Wear eye, hand, face, and clothing protection when using these materials.

Other materials required

The following materials and equipment are required but not supplied:

- Single and/or multichannel pipettes
- Polypropylene tubes (Eppendorf tubes)
- Microplate reader capable of measuring at 540 nm
- Refrigerated centrifuge equipped with microplate swing out carrier
- Paper towels

Critical parameters

A short video showing the assay procedure and critical steps can be found here:
http://youtu.be/wUZZT_L4FQk or on our website:
http://www.quickzyme.com/products/collagen-assay

- It is important to perform the standard preparation and the sample preparation on ice and step 1-10 of the assay procedure below 25°C. If the temperature in the laboratory is higher than 25°C, these steps should also be performed on ice. This is because of the stability of the collagen in samples and standards.
- Thoroughly mixing of sample and dye solution is important. This is done by multiple pipetting up and down. An end-over-end roller can also be used. Do not forget to seal the plate carefully.
- Centrifugation must be carried out in a refrigerated centrifuge at a temperature below 25°C, to prevent denaturation of collagen.
- Removal of the supernatant following centrifugation should be performed very carefully in order to prevent pellets from falling out. Generally the following methods give good results. Put the plate at a 45° angle on a stack of paper towels. If the solution doesn't flow out spontaneously, gently move the plate. If flow starts keep the plate upside down on the towels for a while. Not all the supernatant has to be removed; if some liquid is left this will be removed during the washing step.
- Some sample types give pellets which fall out easily. If this occurs use a multichannel pipet to remove the liquid and be careful not to disturb the pellet. For safety do not try to remove all liquid.
- If washing solution is added using a multichannel pipet, please be careful not to disturb the pellet with the liquid flow in order to prevent loss of the pellet. Generally manual multichannel pipettes give the best results.
- Depending on the sample type pellets could be easily lost during washing. If this occurs use a multichannel pipet to remove the liquid and be careful not to disturb the pellet.

- Serum present in the samples does not affect the assay up to a concentration of at least 10% (v/v) serum.
- If the centrifuge cannot reach 3000 x g, use longer centrifugation times.

Sample preparation

It is important to keep the samples cold, preferably on ice, and always below 25°C . Do not thaw samples in a 37°C water bath, but use a 25°C water bath. Collagen can denature at higher temperatures and denatured collagen is not detected in the assay.

Conditioned culture medium
Conditioned culture medium is collected, and centrifuged for 10 minutes at 1500 x g (at 4°C) to remove cell debris. The supernatant is tested either undiluted or diluted to a proper range using dilution buffer or non-conditioned culture medium (if the culture medium contains serum).
Up to 10% (v/v) serum does not affect the assay.

Cellular extracts
The medium on the cells is removed, and the cells are washed with PBS. Replace the PBS by 0.5 M Acetic acid (250 µl/well of 24-well-plate), incubate o/n at 4°C on a rotating platform, and make a cellular extract using a rubber policeman. The extract is centrifuged 10 minutes 3.000 x g, and the supernatant is tested in the assay in 1-10 fold dilutions made in dilution buffer.

Standard preparation

It is important to perform this procedure on ice.
The collagen standard solution has a concentration of 714 µg/ml (in 20 mM acetic acid). This stock is diluted 10-fold in dilution buffer as followed:,
First add 120 µl Collagen standard solution to 1080 µl dilution buffer, resulting in 1200 µl of the 10-fold dilluted collagen stock (71.4 µg/ml).
The standard curve is built of 10 – 8 – 6 - 4 – 2 - 1 - 0.5 - 0 µg collagen/well. This curve is made by pipetting the following amounts of the 10-fold dilluted collagen stock (71.4 µg/ml) and dilution buffer into Eppendorf tubes (sufficient for 2 standard lines):

10-fold dilluted collagen stock (71.4 µg/ml) (µl)	*dilution buffer (µl)*	*Collagen/well (µg)*
350	0	10
280	70	8
210	140	6
140	210	4
70	280	2
35	315	1
17.5	332.5	0.5
0	350	0

Assay procedure

It is recommended that all samples and standards be assayed in duplicate
It is important to perform step 1-10 below 25°C. *(see also video tutorial on: http://youtu.be/wUZZT_L4FQk)*

1. Prepare the samples as described in 'sample preparation'
2. Prepare the collagen standard as described in 'standard preparation'
3. Pipet 140 µl standard into appropriate wells of the assay microplate (V-shaped plate)
4. Pipet 140 µl dilution buffer into appropriate wells for use as blank
5. Pipet 140 µl of unknown samples (or sample dilutions) into the appropriate wells
6. Add 60 µl dye solution to each well
7. *Mix thoroughly* by pipetting up and down at least five times
   An end-over-end roller can also be used.
8. Seal the plate with the cover seal present in kit.
9. Incubate 10 minutes at room temperature (<25°C), or on ice.
10. Place the assay microplate (V-shaped plate) in the reading microplate (flat bottom plate). Centrifuge this set in a centrifuge equipped with a microplate swing out carrier for 60 minutes at 3000 x g at <25°C, preferably at 4°C (e.g Heraeus Megafuge 1.0R, 4000 rpm). Use a plate set with 200 µl water per well for balance.
    If the centrifuge cannot reach 3000 x g, use a longer centrifugation time.
11. After centrifugation, carefully remove the seal.
12. Do not remove the supernatant, but carefully add 100 µl washing solution to each well.
13. Remove supernatant and washing solution (unbound dye) by placing the plate at a 45° angle on a (5 cm thick) stack of paper towels. If the solution doesn't easily flow out of the plate, gently move the plate. Not all solution has to get out (remaining solution is removed during washing steps). ***Removing the supernatant can be difficult. The pellet (=collagen complex) must not be disturbed !!!***
14. Carefully add 250 µl washing solution to each well and remove this solution by placing the plate at a 45° angle on a stack of paper towels as described above.

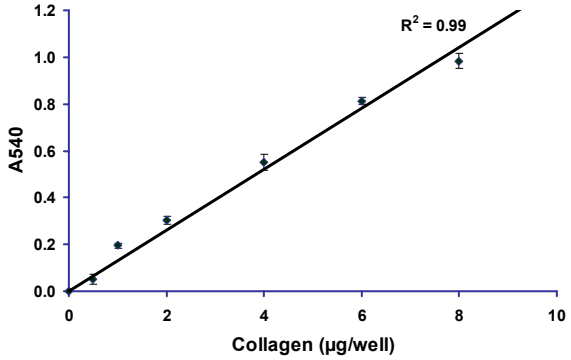
    Do not pipet up and down !!! Please be aware that the liquid flow is not disturbing the pellet. Wells might overflow during this first washing step, this has no influence on the performance of the assay.
15. For a second wash step repeat previous step.
16. Place the plate upside down on a paper towel for at least 5 minutes to remove remainder of wash buffer.
17. Add 150 µl detection solution to the pellets and mix thoroughly by pipetting up and down at least ten times.
18. Transfer 100 µl of the coloured solution to the reading microplate (flat bottom)
19. Read the plate at 540 nm 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. Subtract the average blank. Create a standard curve by plotting the mean A_{540} of each standard on the y-axis against the collagen content on the x-axis (0- 0.5 - 1.0 – 2 – 4 - 6 – 8 - 10 µg collagen/well). Draw a best-fit linearized curve through the points on the graph. Using this standard curve the A_{540} values of the test samples can be calculated to µg collagen/well. From the sample volume used in the assay the collagen concentrations can be calculated.

Typical data

The shown data curve is provided for demonstration only. The exact A_{540} values can vary slightly per experiment.



Typical collagen standard curve in the range
of 0.5 – 10 µg collagen/well (3.5 – 70 µg/ml; blank value ~ 0.090)

Storage conditions

Unopened kit: Store at 4°C. Do not use kit components past kit expiration date.

Opened kit / reconstituted reagents:

- Collagen standard dissolved in 20 mM acetic acid: store at 4°C up to 1 month
- Dye solution: store at 4°C up to 1 month
- Dilution buffer: store at -20°C up to 6 months
- Wash buffer: store at -20°C up to 6 months
- Detection solution: store either at 4°C or room temperature up to 1 month

Related products

- Total Collagen assay
- Sensitive Tissue Collagen assay
- Hydroxyproline assay
- Sensitive Tissue Hydroxyproline assay
- Total Protein assay
- Human MMP-2 activity assay
- Human MMP- 7 activity assay
- Human MMP-8 activity assay
- Human MMP-9 activity assay
- Mouse MMP-9 activity assay
- Human MMP-14 activity assay
- Granzyme B activity Reagent Set
- Human TIMP-1 ELISA
- Human Urokinase (uPA) ELISA

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