QuickZyme

Total Protein Assay

(to be used with acid hydrolyzates)

QuickZyme Biosciences

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

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

Introduction

The QuickZyme total collagen and hydroxyproline assays are frequently used to determine the amount of collagen in biological samples such as tissues, tissue extracts, cell extracts and culture media. For the proper interpretation of the data obtained these should be compared to some reference quantity like wet- or dry- weight of the tissue, amount of protein, DNA etc. Some of these quantities are not, easily to measure, or require an additional sample because they cannot be measured directly in acid hydrolyzates.

It would be helpful if it was possible to determine such a reference in the acid hydrolyzate also used for determination of total collagen or hydroxyproline, such that no longer additional samples or cumbersome methods would be required.

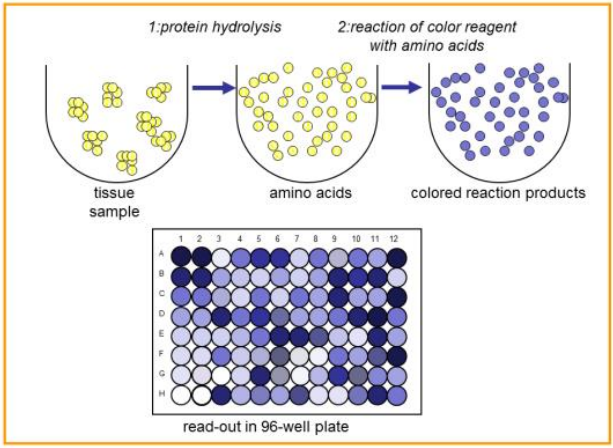
The QuickZyme total protein assay offers a convenient solution in such cases. In this assay the total amount of amino acids present in the same acid hydrolyzate used in the QuickZyme total collagen and hydroxyproline assays is easily determined. This total amount of amino acids in the hydrolyzate is a direct measure of the amount of protein present in the original sample. Combination of the QuickZyme total protein assay with either the total collagen or hydroxyproline assay allows to express data as collagen or hydroxyproline per total amount of protein.

The QuickZyme total protein assay is based on the formation of a blue colored product from genipin with free amino acids (Lee et al. Anal. Chim. Acta 480(2003)267; Fujikawa et al. J. Ferment. Technol. 65(1987)149). The assay measures the total amount of amino acids present in the hydrolyzed sample, with exception of proline and hydroxyproline. The amount of amino acids is a good measure for the amount of protein. A hydrolyzed protein standard is supplied with the kit to directly convert data to mg of protein.

The assay is simple and results in a colored product with an absorbance maximum close to 570 nm. The assay is developed to measure protein in acid tissue and/or protein hydrolyzates in such a way that the assay can be performed directly on the hydrolyzate without a drying or neutralization step.

When used in addition to the QuickZyme Total Collagen, or QuickZyme Hydroxyproline assay kits this assay kit allows for easy expression of the collagen or hydroxyproline amounts per total amount of protein.

Assay principle



What's in the box?

- Adhesive plate seal
- Assay buffer
- Reagent buffer
- Color reagent (in dry form)
- Protein standard (pre-hydrolyzed in dry form)
- 96-well assay plate
- Assay protocol booklet

Other materials required

The following materials and equipment are required but not supplied:

- 6 M HCl for sample and standard dilution
- DMSO for preparing color reagent stock solution
- MilliQ or comparable high quality water
- Single and/or multichannel pipettes
- Incubator for heating at 85^{o}C
- Microplate reader capable of measuring at a wavelength between 540 and 580 nm, 570 nm preferred.
- Micro plate shaker
- Eppendorf tubes

Storage conditions

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

Opened kit / reconstituted reagents:
The reconstituted reagents should be stored light protected at 4°C (< 1 week), longtime storage at -20°C (stable for at least 2 months). The diluted color reagent solution should be prepared fresh and used the same day.

Precaution

Use of the kit requires 6 M HCl and DMSO. See for relevant MSDS: www.quickzyme.com/products/protein assay. Wear eye, hand, face, and clothing protection during hydrolysis of the samples and when using the kit.

Critical parameters

- The samples used in the assay should be present in a solution containing 6 M HCl (acid hydrolyzate).
- The incubation time for color development at 85°C during the last step of the assay is 60 min. in an incubator.

Buffer / reagent preparation

- Assay buffer is ready for use
- Color reagent buffer is ready for use
- Protein standard is present in dry form and needs reconstitution as described in Standard preparation at page 7
- Color reagent is present in dry form and needs reconstitution as described in Color reagent preparation (see below)

Color reagent preparation

Color reagent is present in dry form.

Color reagent stock solution

To prepare color reagent stock solution, add 180 microliter of DMSO to the tube, mix very thoroughly (vortex 2 min. !), wait 30 minutes and mix again (vortex 2 min. !).

Check whether all material is in solution, if not, vortex repeatedly until the material is dissolved. This gives concentrated dye stock solution that is stable for at least 1 week when stored at 4°C in the dark or for at least 2 months when stored at -20°C.

Color reagent working solution
Color reagent working solution is prepared immediately before use by adding 1 volume of color reagent stock solution to 9 volumes of reagent buffer. Mix well. (vortex at least 15 sec.) This solution should be used on the day of preparation and discarded after use.

Sample preparation

The QuickZyme total protein assay was developed to measure protein in acid hydrolyzates, e.g. from conditioned culture medium, cell extracts, tissue homogenates, wet or dry tissue samples and frozen or paraffin-embedded tissue sections (see below). These samples should have been hydrolyzed in 6M HCl (final concentration for acid hydrolysis) 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.
Paraffin tissue sections (e.g. 5 to 10 sections of 10 μm thickness in 150 microliter of 6M HCl) can directly be hydrolyzed, paraffin needs not to be removed. If after hydrolysis a paraffin layer is visible on top of the hydrolyzate, remove a sample of the lower (HCl) layer when the paraffin is still liquid by putting a pipette gently through the upper paraffin layer.

When hydrolyzed samples will need to be diluted (dependent on the sample), dilution should be performed using 6 M HCl.

15 µl of the (diluted) hydrolyzate is used for analysis in the protein assay.

Standard preparation

The protein standard is provided as a dry reagent that needs to be reconstituted with 400 microliter of 6 M HCl (mix well), resulting in a stock solution of 3 mg/ml protein equivalent. The standard is pre-hydrolyzed and can be used as such. The concentrated standard solution can be stored at room temperature and is stable for at least 2 months.

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 6 M HCl according to the scheme below.
This results in a standard line as follows:
3 mg/ml (S1); 1.50 mg/ml (S2);
0.75 mg/ml (S3); 0.375 mg/ml (S4);
0.188 mg/ml (S5); 0.094 mg/ml (S6); 0.047 mg/ml (S7); 0 mg/ml (S8).
Mix all the standards well upon dilution.
15 µl of each standard is used for analysis in the assay. The diluted standard solutions can be used for multiple standard lines and can be stored at 4°C (< 1 week).

Standard label	*Sample from*	*6 M HCl*	*Conc (mg/ml)*
S1	80 µl stock	0	3.00
S2	40 µl S1	40 µl	1.50
S3	40 µl S2	40 µl	0.75
S4	40 µl S3	40 µl	0.375
S5	40 µl S4	40 µl	0.188
S6	40 µl S5	40 µl	0.094
S7	40 µl S6	40 µl	0.047
S8	0 µl	40 µl	0

Pipetting scheme for the preparation of the samples for the protein standard line in duplicate

Assay procedure

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

1. Prepare the color reagent stock solution as described at page 8
2. Prepare the samples as described in 'Sample preparation' at page 9
3. Prepare the protein standard as described in 'Standard preparation'
4. Pipet 15 µl standard into appropriate wells of the assay microplate
5. Pipet 15 µl of each (diluted) sample into the appropriate wells. Depending on the protein content of the sample a dilution step might be required. Dilution should be performed in 6 M HCl
6. Add 120 µl of Assay buffer to each well
7. Mix the contents of the plate by shaking the plate with a plate shaker
8. Prepare a volume of color reagent working solution sufficient for the number of wells to be tested (15 µl/well) by adding 1 volume of color reagent stock solution to 9 volumes of reagent buffer and mix well (resp 1.5 µl + 13.5 µl/well)(vortex 15 sec.)
9. Add 15 µl of color reagent working solution to each well
10. Cover the plate with an enclosed adhesive plate seal (remove white part of seal completely)
11. Mix well by shaking the plate. Incubate 60 minutes at 85°C in an incubator
12. Cool the plate to room temperature
13. Clean the bottom of the plate, remove the seal 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 protein 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 protein concentration can be calculated manually as follows:

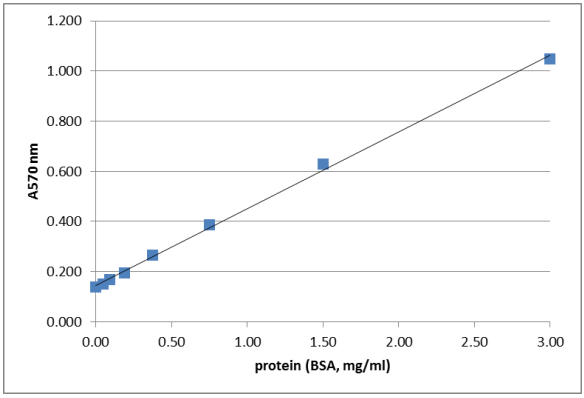
- Average the duplicate readings for each standard or sample and subtract the average blank from all readings.

- Create a standard curve by plotting the mean A_{570} (minus blank) of each standard on the y-axis against the protein content on the x-axis (0 - 0.047 - 0.094 - 0.188 - 0.375 - 0.75 - 1.50 - 3.00 mg/ml protein). Draw a best-fit linearized curve through the points on the graph. Use this standard curve to convert the A_{570} – blank values of the test samples to mg/ml protein. This gives the concentration in the hydrolyzate. If after hydrolysis a dilution step is included, the concentration should be

multiplied with the dilution factor to give the protein concentration in the hydrolyzate. Depending on the sample preparation the amount of protein in the original samples can be calculated.

Typical data

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



A typical protein standard curve in the range of 0.047-3.00 mg/ml protein, the background is not subtracted).

Related products

- Total Collagen assay
- Sensitive Tissue Collagen assay
- Hydroxyproline assay
- Sensitive Tissue Hydroxyproline assay
- Soluble Collagen 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

QuickZyme Biosciences B.V.

Zernikedreef 9
2333 CK Leiden
The Netherlands

www.quickzyme.com

E-mail info@quickzyme.com
Phone +31888666114
+31888666024