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Thiol Quantification kit

Applicable to:

418-0002

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Introduction

This Thiol Quantification assay kit is designed to be dual purpose so two protocols are provided to prepare the calibration curve. Step 2 has been optimized to quantify the amount of thiol/sulfhydryl (-SH) groups in samples of thiolated antibodies or proteins (detection range 0.5 – 25 µM). Step 3 has been designed as a more general thiol detection kit (detection range 5 – 250 µM). Select required protocol based on the expected level of thiols. The assay is in a convenient 96-well plate format and contains all necessary reagents, including standards. This assay system utilises a colorimetric reagent, DTNB. The product of the thiol/DTNB interaction is yellow and results are obtained in 5 minutes by measuring the absorbance at either 405 nm or 412 nm.

Kit contents and storage

Components in the 2-plate Thiol Quantification assay kit

Store at 2-8°C:
1 x 5 ml of 50x DTNB (thiol detector)
1 x 15 ml of 10x reaction buffer

Store at -20 or -70°C:
1 vial of lyophilized cysteine (standard)

Store at room temperature:
2 x 96-well plates

Instructions

Overview of Thiol Quantification assay kit

(1) Dilute the 10x reaction buffer to 1x.

(2) and (3) Reconstitute the lyophilized standard and prepare its dilutions for the calibration curve.

(4) Load 200 µl per well of each standard dilution in triplicate.

(5) Load 200 µl per well of sample in triplicate.

(6) Dilute the 50x DTNB to 5x.

(7) Start the reaction loading 50 µl per well of 5x DTNB and read the absorbance after 5 minutes.

(8) Analyze data.

Reagent preparation considerations

The test sample must have had any remaining thiolation agent removed before conducting the assay (see FAQs). The sample buffer the samples are in must be ≤0.1M at pH 6.5-8.5.
The buffer must be free of reducing agents in test samples (e.g. DTT, β-mercaptoethanol or mercaptoethylamine).
Ensure the supplied reagents are fully equilibrated to room temperature before use.

Detailed assay procedure

1. **Reaction buffer preparation.** Dilute the reaction buffer by a factor of 10, using deionized water (e.g. add 1 ml of 10x reaction buffer to 9 ml of water). Once diluted, the 1x reaction buffer can be stored at 2-8°C.

2. **Standard preparation for use with thiolated antibodies/ proteins (detection range 0.5 µM – 25 µM)**

   2.1. Cysteine standard reconstitution. Reconstitute the lyophilized cysteine to a 10 mM solution by adding 400 µl of deionized water and mixing gently.

   2.2. Preparation of cysteine standard dilutions. Dilute the reconstituted cysteine to 1 mM by adding 10 µl of the 10 mM stock to 90 µl of 1x reaction buffer. With the rest of the 10 mM stock make 50 µl aliquots and store

them at -20°C (NOTE: the 1 mM standard cannot be stored). Starting from 1 mM cysteine, prepare the serial dilutions of standard as per Table 1.

Table 1. 0.5 µM – 25 µM calibration curve dilutions

Thiols (µM)	Cysteine (µl)	1x Reaction Buffer (µl)
25.00	35	1365
12.50	700	700
6.25	700	700
3.13	700	700
1.56	700	700
0.78	700	700
0.39	700	700
0.00	0	700

3. **Standard preparation for use with other thiolated compounds (detection range 5 µM – 250 µM)**

   3.1. Cysteine standard reconstitution. Reconstitute the lyophilized cysteine to a 10 mM solution by adding 400 µl of deionized water and mixing gently.

   3.2. Preparation of cysteine standard dilutions. Starting from the 10 mM cysteine, prepare the serial dilutions of standard as per Table 2. Make 50 µl aliquots with the rest of 10 mM cysteine standard and store them at -20°C.

Table 2. 5 µM – 250 µM calibration curve dilutions

Thiols (µM)	Cysteine (µl)	1x Reaction Buffer (µl)
250.00	35	1365
125.00	700	700
62.50	700	700
31.25	700	700
15.63	700	700
7.81	700	700
3.91	700	700
0.00	0	700

4. **Standard loading.** Load into the plate 200 µl per well of each standard in triplicate.

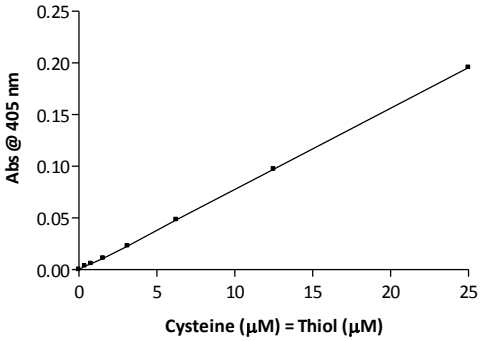
5. **Sample loading.** Load into the plate between 20 µl to 200 µl of sample. If sample volume is <200 µl add reaction buffer to make a total test volume of 200 µl per well. It is recommended to load the sample in triplicate.

6. **Thiol detector preparation.** Dilute by a factor of 10 the thiol detector (e.g. 100 µl 50x DTNB in 900 µl of 1x reaction buffer) in order to have 5x DTNB. Once diluted the DTNB reagent is not stable.

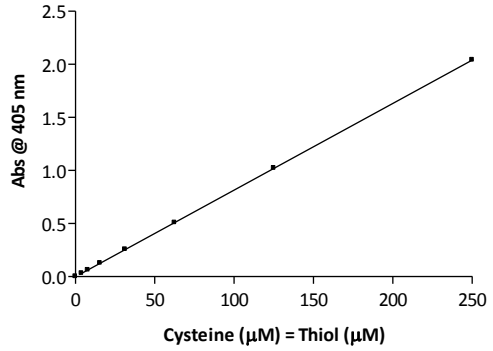
7. **Thiol detection assay run.** Load 50 µl per well of 5x DTNB to each well containing a cysteine standard or sample. The DTNB reagent is at 1x concentration in the test well. Incubate the plate for 5 minutes with gentle shaking. Longer incubation times of up to 1 hour have no negative effect on the result. Measure the absorbance at 405 nm or 412 nm.

8. **Data analysis.** Subtract the average reading for the 0 µM cysteine standard (Blank) from each of the other assay wells. Plot the cysteine standard curve; absorbance versus µM cysteine. Calculate test sample thiol/sulfhydryl content using standard curve; note µM cysteine = µM thiol.

Typical 0.5 µM – 25 µM standard curve



Typical 5 µM – 250 µM standard curve



Related products

Description	Product Code
Thiolation kit	419-0005
Maleimide-HRP	401-0005
Maleimide-Alk Phosphatase	402-0005
Maleimide-R Phycoerythrin	403-0005
Maleimide-Allophycocyanin	404-0005
Maleimide-Streptavidin	405-0005
Maleimide-Ovalbumin	407-0005
Maleimide-BSA	408-0005
Maleimide-KLH	409-0005
KLH Immunogen Kit (for sulfhydryls)	460-0500
Ovalbumin Immunogen Kit (for sulfhydryls)	461-0500
BSA Immunogen Kit (for sulfhydryls)	462-0500
40 nm InnovaCoat GOLD – Maleimide	270-0015
20 nm InnovaCoat GOLD – Maleimide	271-0015

FAQs

When and why would you quantify the number of free thiols in a sample?

Quantifying the number of free thiols in a sample can be useful in a number of applications. For example, if the final purpose is the conjugation of a thiolated molecule to a maleimide – activated label, quantifying the number of free thiols on the molecule is highly recommended in order to have a more controlled conjugation reaction.

What is the minimum thiol concentration detected with the assay?

A concentration of 1.5 µM of thiols can be easily detected.

How do I remove the thiolating agent before testing?

Dialysis of thiolated antibodies/ proteins is not recommended due to the consequent big dilution. The thiolating reagent can be removed using a small size-exclusion/gel filtration gravity or spin column. Consult manufacturers' instructions for use and select resin for your needs.

My buffer is outside the recommended ranges what should I do?

If the buffer is too concentrated, dilute the sample to within buffer range. If the sample cannot be diluted further, the buffer could alternatively be exchanged using a size-exclusion/gel filtration gravity or spin column.

What if the measured absorbance of the sample is out of the range set by the calibration curve?

Concentrate or dilute the sample in order to be within the linear range of the calibration curve.

For further information about Innova's products please see our website:
https://www.innovabiosciences.com

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