

Thiol Quantification Kit

Applicable to: 418-0002

Release 2 © EXPEDEON. 18/12/2018

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 μ M). Step 3 has been designed as a more general thiol detection kit (detection range 5 – 250 μ M). 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

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

- Dilute the 10x reaction buffer to 1x.
- and (3) Reconstitute the lyophilized standard and prepare its dilutions for the calibration curve.
- Load 200 μ l per well of each standard dilution in triplicate.
- Load 200 μ l per well of sample in triplicate.
- Dilute the 50x DTNB to 5x.
- Start the reaction loading 50 μ l per well of 5x DTNB and read the absorbance after 5 minutes.
- 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 ≤ 0.1 M 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

- 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 μ M – 25 μ M)

- Cysteine standard reconstitution.** Reconstitute the lyophilized cysteine to a 10 mM solution by adding 400 μ l of deionized water and mixing gently.
- Preparation of cysteine standard dilutions.** Dilute the reconstituted cysteine to 1 mM by adding 10 μ l of the 10 mM stock to 90 μ l of 1x reaction buffer. With the rest of the 10 mM stock make 50 μ l 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 μ M – 25 μ M calibration curve dilutions

| Thiols (μ M) | Cysteine (μ l) | 1x Reaction Buffer (μ l) |
|-------------------|---------------------|-------------------------------|
| 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 μ M – 250 μ M)

- Cysteine standard reconstitution.** Reconstitute the lyophilized cysteine to a 10 mM solution by adding 400 μ l of deionized water and mixing gently.
- Preparation of cysteine standard dilutions.** Starting from the 10 mM cysteine, prepare the serial dilutions of standard as per Table 2. Make 50 μ l aliquots with the rest of 10 mM cysteine standard and store them at -20°C.

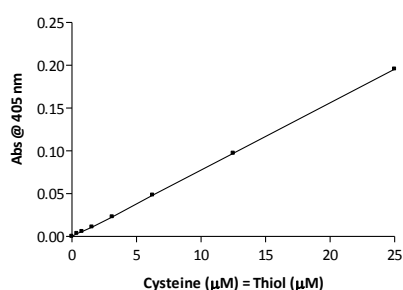
Table 2. 5 μ M – 250 μ M calibration curve dilutions

| Thiols (μ M) | Cysteine (μ l) | 1x Reaction Buffer (μ l) |
|-------------------|---------------------|-------------------------------|
| 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 |

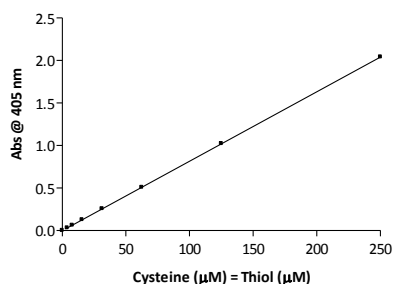
- Standard loading.** Load into the plate 200 μ l per well of each standard in triplicate.
- Sample loading.** Load into the plate between 20 μ l to 200 μ l of sample. If sample volume is < 200 μ l add reaction buffer to make a total test volume of 200 μ l per well. It is recommended to load the sample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

6. **Thiol detector preparation.** Dilute by a factor of 10 the thiol detector (e.g. 100 μ l 50x DTNB in 900 μ l 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 μ l 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 μ M cysteine standard (Blank) from each of the other assay wells. Plot the cysteine standard curve; absorbance versus μ M cysteine. Calculate test sample thiol/sulfhydryl content using standard curve; note μ M cysteine = μ M thiol.

Typical 0.5 μ M – 25 μ M standard curve



Typical 5 μ M – 250 μ M standard curve



RELATED PRODUCTS

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

For information about other conjugation related products see www.expedeon.com

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 μ M 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.

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www.expedeon.com

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: www.expedeon.com/contact/

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