



## Protein Thiolation Kit

419-0002 1 vial (for 2mg activation)  
Release 1 08/03/2016

419-0005 1 vial (for 5mg activation)

### Introduction

The Protein Thiolation Kit can be used for most proteins, allowing activation simply by adding a protein solution to a lyophilised thiolation reagent. Designed to facilitate conjugation of proteins to Maleimide-Activated labels.

### Shipping and storage

This kit is shipped at ambient temperature. Upon receipt, store the Freezer Pack at -20°C, and the Room Temperature Pack at room temperature. Allow the freezer pack to thaw to room temperature before opening.

### Protein Concentration and Quantity

The Thiolation kits are designed to activate 2mg or 5mg of protein in 2ml or 5ml of a suitable buffer, respectively (depending on kit size). The protein to be activated must be purified and at a concentration of 1 mg/ml. Higher protein concentrations should be diluted to 1 mg/ml with Protein Resuspension Buffer. If your protein is less than 1 mg/ml, or is not in a suitable buffer (please see the section below regarding buffer considerations), please contact our Technical Support Team.

### Buffer considerations

Buffer components	Protein buffer
pH	7-9
Amine free buffer (ideally phosphate buffer)	✓
Non-buffering salts (e.g. sodium chloride)	✓
Chelating agents (e.g. EDTA)	✓
Sugars	✓
Glycerol	<50%
Thimerosal/ Thiomerosal	✗
Merthiolate	✗
Sodium Azide*	<0.1%
Tris	<20mM
Glycine	✗
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	✗

*\*Please note that individually the concentrations shown should not affect the reaction. However in combination with additional compounds that are not recommended above a certain concentration, the reaction may be affected.*

### Kit contents

#### Thiolation Kit

- 1 glass vial of Thiolation Reagent
- 1 bottle of Clean-Up Reagent\*
- 1 bottle of Protein Resuspension Buffer

#### Thiolation Detection Kit

- 1 tube of Thiol Detection Reagent
- 1 tube of Thiol Detection Buffer

*\* Warm the Clean-Up Reagent by placing the tube in warm water (not warmer than 40°C) for 10 minutes, then shaking to re-dissolve the contents. Once dissolved, maintain the tube at ~22°C to prevent any crystal formation before use.*

### Instructions

#### Setting up your thiolation reaction

##### 1. Protein Thiolation.

Add 2ml or 5ml of the 1 mg/ml protein into the Thiolation Reagent vial (depending on kit size). Mix gently and incubate for 1 hour at room temperature.

##### 2. Thiolated Protein Purification

- a. Warm the Clean-Up Reagent by placing the tube in warm water (not warmer than 40°C) for 10 minutes and mixing regularly. Make sure any crystals have been re-dissolved, shake if necessary.
- b. Add 2ml or 5ml of Clean-Up Reagent (depending on kit size) to the thiolated protein, at a 1:1 ratio. Mix and incubate at room temperature or on ice for 20 minutes.
- c. Centrifuge for 10 minutes at 15,000g.
- d. Remove sample from the centrifuge taking care not to dislodge the small pellet at the bottom of the tube. If no pellet is seen add another 2ml or 5ml (depending on kit size) of Clean-Up Reagent, mix well and incubate on ice for a further 10 minutes and centrifuge. Repeat this step if necessary.
- e. Carefully remove the supernatant and store until efficient precipitation has been confirmed.
- f. Add 2ml or 5ml of Protein Resuspension Buffer (depending on kit size) to the pellet and resuspend gently.
- g. The thiolated protein is now ready to use.

## Storage of activated material

The activated protein should be stored on ice. It is very reactive and should be used within 1 hour. The activated protein is not stable enough for long term storage.

## Qualitative Thiol Detection Test (optional)

Each Thiolation Kit is supplied with a Thiolation Detection Kit. These reagents are provided as a positive test for protein thiolation in order to give the option of confirming the thiolation chemistry is working. In this test, a positive result is detected by the development of a yellow color, which is visible by eye. There are two methods by which an absolute reading can be obtained.

### Thiolation Detection by Plate Reader

1. Dilute the Thiol Detection Reagent 1:10 with Thiol Detection Buffer (i.e. 100µl Thiol Detection Reagent + 900µl Thiol Detection Buffer).
2. Test well: Add 200µl thiolated protein to a blank well and 50µl of the diluted Thiol Detection Reagent.  
Control well: Add 200µl Protein Resuspension Buffer to a blank well and 50µl of the diluted Thiol Detection Reagent.
3. After 5 minutes, read the well at 405nm.
4. A yellow color will develop in the test well compared to the control well, this is visible by eye and will develop more over time. After 5 minutes incubation, an approximate absorbance reading of 0.10 should be expected for the control well. The test values will vary depending on the protein, but approximate absorbance readings of  $\geq 0.20$  should be expected for antibodies and  $\geq 0.25$  for BSA.

### Thiolation Detection by Spectrophotometer

1. Dilute the Thiol Detection Reagent 1:10 with Thiol Detection Buffer (i.e. 100µl Thiol Detection Reagent + 900µl Thiol Detection Buffer).
2. Control sample: 200µl Protein Resuspension Buffer + 50µl diluted Thiol Reagent.  
Test Sample: 200µl thiolated protein + 50µl diluted Thiol Reagent.
3. At 405 nm, blank the spectrophotometer using Protein Suspension Buffer.
4. After 5 minutes read the Control sample and then the Test sample. A yellow color will develop in the test sample compared to the control sample, this is visible by eye and will develop more over time. An approximate OD of 0.10 should be expected for the control sample. The test values will vary depending on the protein, but approximate OD of  $\geq 0.30$  should be expected for Antibodies and  $\geq 0.30$  for BSA.

***This test is provided as a qualitative test to show a positive thiolation result. Our Thiol Detection Kit is available to help determine quantitative results.***

## Generation of Conjugates

This kit has been designed to generate conjugates with Maleimide-activated labels.

2mg or 5mg of thiolated protein can be used 1:1 with any maleimide-activated label of choice, following conjugation protocol.

## FAQs

Please view our website for FAQs:  
[www.innovabiosciences.com](http://www.innovabiosciences.com)

## Technical Support

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E-mail: [technical.enquiries@innovabiosciences.com](mailto:technical.enquiries@innovabiosciences.com)

## Related Products

418-0002	Thiol Detection Kit
401-0005	Maleimide-HRP
402-0005	Maleimide-Alkaline Phosphatase
403-0005	Maleimide-R Phycoerythrin
404-0002	Maleimide-Allophycocyanin-XL
405-0005	Maleimide-Streptavidin
407-0005	Maleimide-Ovalbumin
408-0005	Maleimide-BSA
409-0005	Maleimide-KLH
425-0300	Thunder-Link® PLUS Oligo Conjugation System
270-0015	InnovaCoat GOLD Maleimide 200D 40nm Gold Conjugation Kits
271-0015	InnovaCoat GOLD Maleimide 200D 20nm Gold Conjugation Kits

***For information about other conjugation related products see [www.innovabiosciences.com](http://www.innovabiosciences.com)***

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