

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/sb11.pdf>

**General Information**

Modification of the thiol residues of proteins is one of the post-translational modifications and it occurs according to the redox states within cells. It has recently been revealed that the modifications of thiol residues control cellular events such as transcription, protein expression, cell death, etc. In this regard, detection of the redox states of individual thiol residues in a protein is important to understand cellular events.

This kit enables visualization of the redox states of thiol residues in proteins by electrophoretic analysis. Protein-SHifter, a component of this kit, has a maleimide group and can bind covalently to a thiol residue of a protein. A mobility shift corresponding to about 15 kDa of molecular mass is observed on the electrophoretic analysis when one molecule of Protein-SHifter binds to that of thiol of the target protein. Thus, the number of thiol groups in protein can be clearly identified by SDS-PAGE through the use of the mobility shift assay.

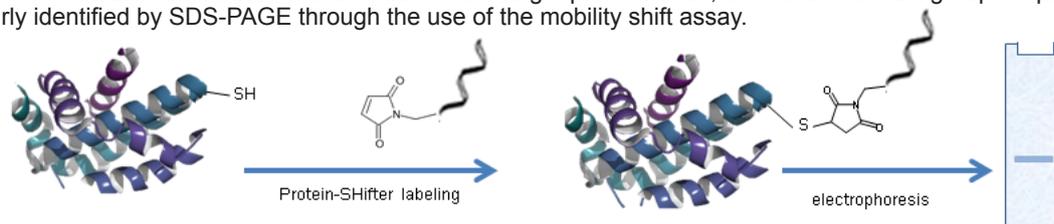


Figure 1 Mechanism of thiol modification with Protein-SHifter

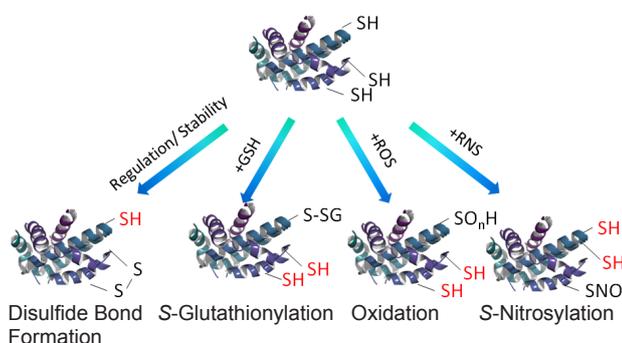


Figure 2 Modification profiles of thiol residues in a protein

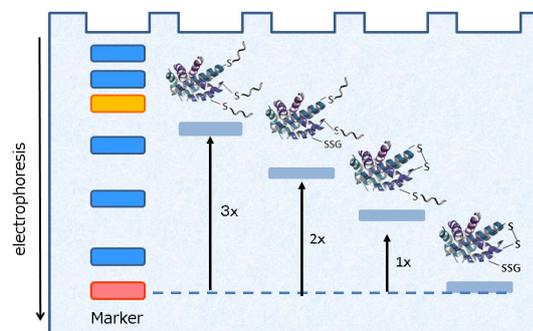


Figure 3 Visualization of redox states of thiol residues in a protein

A mobility shift corresponding to about 15 kDa is observed when one molecule of Protein-SHifter binds to that of thiol of a protein. (The mobility shift changes according to conditions such as the kinds of protein and the number of Protein-SHifter bound, etc.)

**Kit Contents**

- Protein-SHifter x 5
- Reaction Buffer A x 1
- Reaction Buffer B x 1

**Storage Condition**

Store at 0 - 5°C

**Required Equipment and Materials**

- 10 µl and 100-200 µl pipettes
- Electrophoresis: Gel, Loading Buffer, Protein Staining (CBB: Coomassie Brilliant Blue etc.)
- Incubator (37°C)
- PBS: for sample dilution

**Precaution**

- The content in Protein-SHifter is a mobile white solid. Centrifuge the tube briefly before opening the cap because the content may be on the tube wall or on the ceiling of the cap.
- This kit is designed for electrophoretic experiments. Samples should preferably be purified proteins. If you use cell lysates for western blotting, we recommend use of other Dojindo products, *-SulfoBiotics- Protein Redox State Monitoring Kit Plus* (Product code: SB12).
- Reaction Buffer B may cause precipitation. Warm it at 40-50°C to dissolve the precipitate.

**Sample preparation**

1. Add 4 µl of Reaction Buffer A to Protein-SHifter.
2. Add 2 µl of the sample solution to the solution of Step 1 and mix by pipetting.
  - ※ The suitable concentration of protein and thiol residue to obtain the best results is as follows; proteins: 0.1 - 1 mg/ml, thiol residues: < 100 µmol/l.
3. Add 4 µl of Reaction Buffer B to the solution of Step 2 and mix by pipetting.
  - ※ Warm the solution to completely dissolve a precipitate if precipitation occurs.
4. Incubate at 37°C for 30 minutes.
  - ※ Proceed to Step 5 immediately.
5. Apply the sample of Step 4 to a gel for electrophoretic analysis.
6. Visualize the separated protein by CBB staining.

## Detection of Redox State of Thiols residues in GAPDH (Glyceraldehyde 3-phosphate dehydrogenase)

1. GAPDH solution (1 mg/ml, 10  $\mu$ l) was added to a 1.5 ml tube. DTT (dithiothreitol) solution (100 mmol/l, 1  $\mu$ l) was then added to the tube and mixed by pipetting.
2. The tube was incubated at 37°C for 10 minutes.
3. The solution at Step 2 was transferred to a 10K filtration tube, which was then centrifuged at 7,500 x g for 15 minutes.
4. TE Buffer ([50 mmol/l Tris-HCl (pH 7.5), 1 mmol/l EDTA], 100  $\mu$ l) was added to the tube at Step 3, which was then centrifuged at 7,500 x g for 15 minutes.
5. Step 4 was repeated.
6. TE Buffer (50  $\mu$ l) was added to the tube at Step 5 and was mixed by pipetting. (0.2 mg/ml GAPDH)
7. Reaction Buffer A (4  $\mu$ l) was added to Protein-SHifter and was mixed by pipetting.
8. GAPDH solution (0.2 mg/ml, 2  $\mu$ l) was added to the tube at Step 7 and was mixed by pipetting.
9. Reaction Buffer B (4  $\mu$ l) was added to the tube at Step 8 and was mixed by pipetting.
10. The tube at the Step 9 was incubated at 37°C for 30 minutes.
11. Loading Buffer ([10 (w/v) % sodium dodecyl sulfate, 50 (v/v) % glycerol, 0.2 mol/l Tris-HCl (pH 6.8) , 0.05 (w/v) % bromophenol blue], 2  $\mu$ l) was added to the tube at Step 10 and was mixed by pipetting.
12. The solution at Step 11 was used for electrophoresis.
13. The protein was visualized by CBB staining.

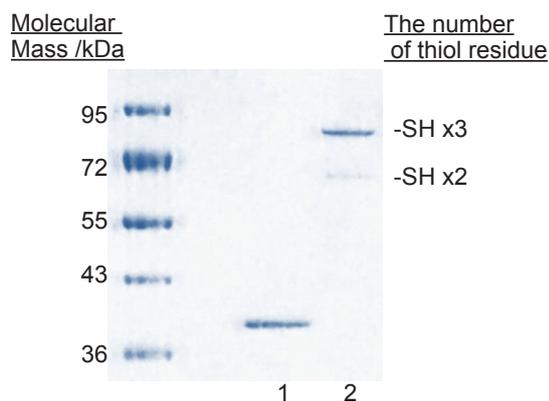


Figure 4 Detection of the number of thiols residues in GAPDH

- Lane 1: GAPDH, Lane 2: Labeled GAPDH
- 15% SDS-polyacrylamidegel

## Reference

- 1) Satoshi Hara, Tatsuya Nojima, Kohji Seio, Masasuke Yoshida, Toru Hisabori, "DNA-maleimide: An improved maleimide compound for electrophoresis-based titration of reactive thiols in a specific protein", *Biochim. Biophys. Acta*, **2013**, 1830(4) 3077.
- 2) Satoshi Hara, Yuki Tatenaka, Yuya Ohuchi, Toru Hisabori, "Direct determination of the redox status of cysteine residues in proteins *in vivo*", *Biochem. Biophys. Res. Commun.*, **2015**, 456(1) 339.

If you need more information, please contact Dojindo technical service.

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