-SulfoBiotics-
Protein Redox State Monitoring Kit

Technical Manual

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

General Information

Modification of protein thiols is one of the most important post-translational modifications and it occurs according to the redox states in cells. It has recently been revealed that the modifications of thiol groups control cellular functions such as transcription, protein expression, cell death, *etc*. Therefore, detection of the redox states of the individual protein is important to understand cellular events.

-SulfoBiotics- Protein Redox State Monitoring Kit enables visualization of the redox states of protein by electrophoretic analysis. Protein-SHifter, one of the components in the kit, has a maleimide group that can bind covalently to a protein thiol group. A mobility shift corresponding to about 15 kDa of molecular mass is observed by the electrophoretic analysis when one molecule of Protein-SHifter binds to a thiol group of the target protein. Thus, the number of free thiol groups on a protein can be clearly identified by SDS-PAGE through the mobility shift assay.

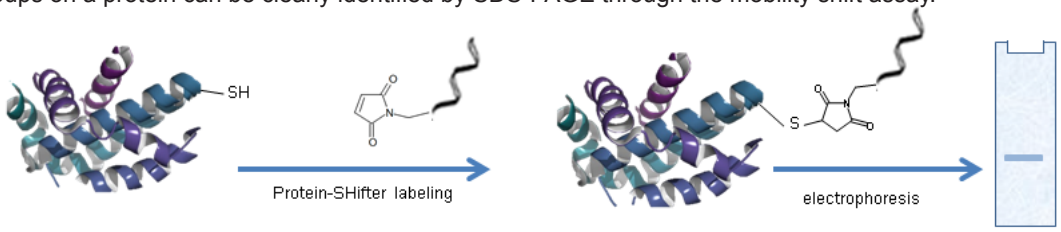

Figure 1 Schematic protocol of Protein Redox State Monitoring Kit

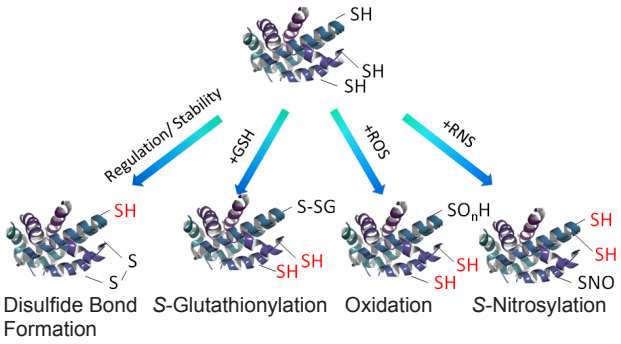

Figure 2 Representative protein thiol modifications

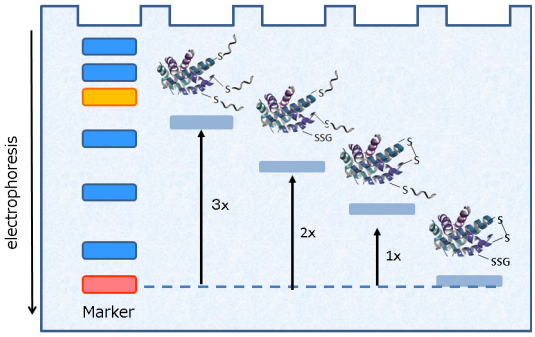

Figure 3 Visualization of protein redox states

A mobility shift corresponding to about 15 kDa is observed when one molecule of Protein-SHifter binds to a thiol group of a protein.

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

- 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 to use the following product,
  –*SulfoBiotics-* Protein Redox State Monitoring Kit Plus (Product code: SB12).
- If a precipitate is observed in Reaction Buffer B, warm it at 40-50°C to dissolve it.

General Protocol

1. Add 4 µl of Reaction Buffer A to a tube of Protein-SHifter.
   ※ Protein-SHifter is unstable in Reaction Buffer A,B. Proceed to Step 2 immediately after adding Reaction Buffer A.
2. Add 2 µl of the sample solution to the solution of Step 1 and mix by pipetting.
   ※ The concentration of proteins and thiols should be as follows; proteins: 0.1 - 1 mg/ml, thiols: < 100 µmol/l.
   ※ Visit our website (SB11 Q&A) or contact our technical support for information about the way to determine the concentration of thiols in your sample.
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.
   ※ Defoam by centrifugation at 7,000 x *g* for 1 - 2 minutes if foaming occurs.
4. Incubate at 37°C for 30 minutes.
   ※ Proceed to Step 5 immediately.
5. Add a Loading buffer to the solution of Step 4 and apply the solution to gel electrophoresis.
   ※ This kit does not contain Loading buffer. When using (5x) Loading buffer, add 2 µl of (5x) Loading buffer to 10 µl of the solution of Step 4 and apply all of the solution to the gel.
6. Detect the protein by CBB staining.

Usage Example

Analysis of Redox State of GAPDH (Glyceraldehyde 3-phosphate dehydrogenase)

1. GAPDH solution (1 mg/ml, 10 µl) was added to a 1.5 ml tube. DTT (dithiothreitol) Solution (100 mmol/l, 1 µl) was then added to the tube and mixed by pipetting.
2. The tube was incubated at 37℃ for 10 minutes.
3. The solution of Step 2 was transferred to a 10K filtration tube and the tube was centrifuged at 7,500 x *g* for 15 minutes.
4. TE Buffer ([50 mmol/l Tris-HCl (pH 7.5), 1 mmol/l EDTA], 50 µl) was added to the tube of Step 3 and the tube was centrifuged at 7,500 x *g* for 15 minutes.
5. Step 4 was repeated.
6. TE Buffer (50 µl) was added to the tube of Step 5 and was mixed by pipetting to recover the protein (0.2 mg/ml GAPDH).
7. Reaction Buffer A (4 µl) was added to Protein-SHifter and was mixed by pipetting.
8. The GAPDH solution (0.2 mg/ml, 2 µl) of Step 6 was added to the tube of Step 7 and was mixed by pipetting.
9. Reaction Buffer B (4 µl) was added to the tube of Step 8 and was mixed by pipetting.
10. The tube of the Step 9 was incubated at 37℃ 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 µl) was added to the tube of Step 10 and was mixed by pipetting.
12. The solution of Step 11 was used for electrophoresis.
13. The protein was detected by CBB staining.

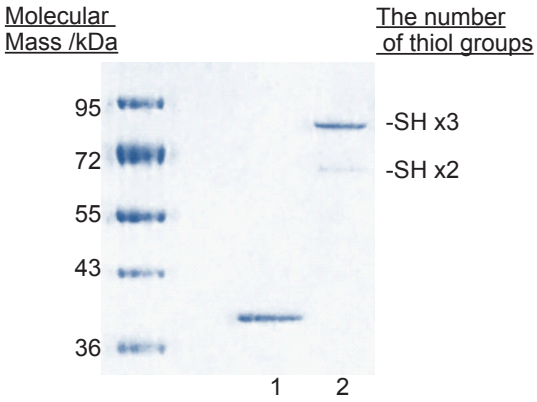

Figure 4 Visualization of the redox state of the GAPDH

- Lane 1: GAPDH, Lane 2: Labeled GAPDH
- 15% SDS-polyacrylamide gel

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.