-SulfoBiotics-
Protein Redox State Monitoring Kit Plus Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/sb12.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 modification of thiol residue(s) controls cellular events such as transcription, protein expression, cell death, etc. In this regard, detection of the redox states of individual thiol residue(s) in a protein is important in understanding cellular events. This kit enables visualization of the redox states of thiol residues in proteins electrophoretically.

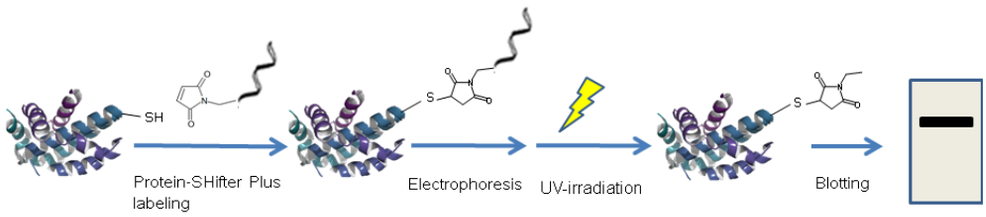

Figure 1 Protein separation with Protein-SHifter Plus and transblotting

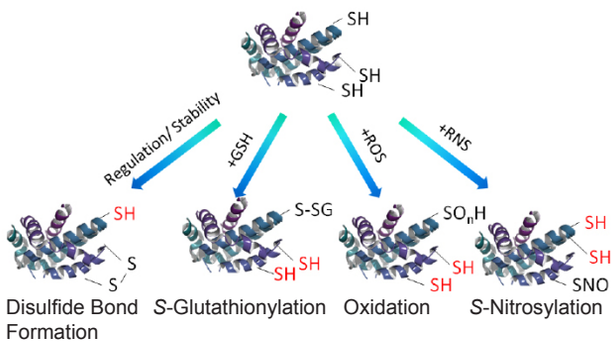

Figure 2 Modification profiles of thiol residues in a protein

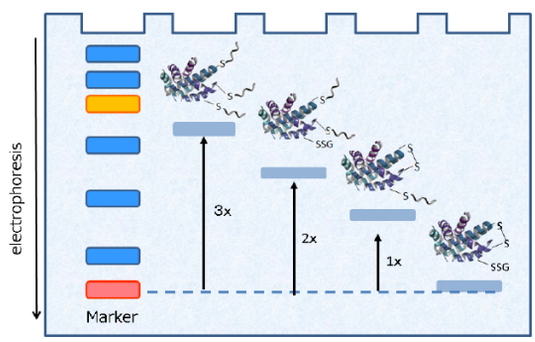

Figure 3 Visualization of redox states of thiol residues in a protein

Kit Contents

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

Storage Condition

Store at 0 - 5 °C

Required Equipment and Materials

- 10 µl and 100-200 µl pipettes
- Incubator (37°C)
- Electrophoresis: Gel, Loading Buffer
- Centrifuge
- Westernblot: Transfer system, PVDF (polyvinyl difluoride) membrane
- Transilluminator
- 10 (w/v) % TCA (Trichloroacetic acid) solution
- Acetone
- 70 (v/v) % EtOH solution
- PBS: for sample dilution

Precaution

- The content in Protein-SHifter Plus 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.
- Reaction Buffer B may have precipitates at the bottom. If this happens, incubate Reaction Buffer B at 40-50°C until the precipitates are dissolves.

Sample preparation

For adherent cells

1. Prepare 5 - 10x10^5 cells for the assay.
   ※ The appropriate range of the protein concentration is between 0.1 and 1 mg/ml.
   ※ Protein-SHifter Plus might not sufficiently label the thiol residues of protein if the concentration is over 1 mg/ml.
2. Aspirate the medium and wash the cells twice with cold 500 µl PBS.
3. Add 500 µl of cold 10 % TCA solution to each well and incubate the plate on ice for 30 minutes.
4. Scrape the cells from the wells and transfer them to a tube.
5. Centrifuge at 1,000 x *g* for 3 minutes and aspirate the supernatant.
6. Add 500 µl of cold acetone to each tube and centrifuge 1,000 x *g* for 3 minutes and aspirate the supernatant twice.
7. Add 500 µl of cold 70% EtOH solution to each tube and centrifuge 1,000 x *g* for 3 minutes and aspirate the supernatant.
8. Add 100 µl of Lysis Buffer (Add protease inhibitor, when needed.) to each tube and dissolve the cell pellet by sonication.
   ※ If the gelation of the solution happens, dilute it with Lysis Buffer.
   ※ The sample should be applied for electrophoretic analysis immediately.

For non-adherent cell

1. Prepare 5 - 10x10^5 cells/tube for the assay.
   ※ The appropriate range of the protein concentration is between 0.1 and 1 mg/ml.
   ※ Protein-SHifter Plus might not sufficiently label the thiol residues of protein if the concentration is over 1 mg/ml.
2. Centrifuge at 1,000 x *g* for 3 minutes and aspirate the supernatant.
3. Add 500 µl of PBS to each tube and centrifuge 1,000 x *g* for 3 minutes and aspirate the supernatant .
4. Repeat step 3.
5. Add 150 µl of cold 10 % TCA solution to each tube and incubate the tube on ice for 30 minutes.
6. Centrifuge at 1,000 x *g* for 3 minutes and aspirate the supernatant.
7. Add 500 µl of cold acetone and centrifuge at 1,000 x *g* for 3 minutes and aspirate the supernatant twice.
8. Add 500 µl of cold 70% EtOH solution and centrifuge at 1,000 x *g* for 3 minutes and aspirate the supernatant.
9. Add 100 µl of Lysis Buffer (Add protease inhibitor, when needed.) and dissolve the cell pellet by sonication.
   ※ If the gelation of the solution happens, dilute it with Lysis Buffer.
   ※ The sample should be applied for electrophoretic analysis immediately.

Labeling Procesure

1. Add 4 µl of Reaction Buffer A to Protein-SHifter Plus.
2. Add 2 µl of the sample solution to the solution at Step1 and mix by pipetting.
3. Add 4 µl of Reaction Buffer B to the solution at Step2 and mix by pipetting.
   ※ If precipitates form when mixing, incubate the solution at 40-50°C until it redissolves.
4. Incubate at 37°C for 30 minutes.
   ※ The sample should be applied for electrophoretic analysis immediately.
   ※ Before blotting, expose the gel to UV-rays with a transilluminator for 10 minutes.

Usage Example

Detection of Redox State of GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) in the HeLa cell

1. HeLa cells were seeded to the concentration of 5 x 10^5 cell/well on a 6-well plate and cultured overnight at 37 °C in a 5%-CO_2 incubator.
2. The cells were washed using cold 500 µl PBS, and Diamide [1,1'-Azobis(*N,N*-dimethylformamide)] or H_2O_2 (1 mmol/l, 500 µl) were added to the culture cell.
3. The cells were then incubated at 37 °C for 15 minutes.
4. Following aspiration of the supernatant, TCA solution (10 %, 500 µl) was added to each well.
5. The plate was incubated on ice for 30 minutes.
6. The cells was scraped and transferred to tube.
7. The supernatant was removed by centrifugation at 1,000 x *g* for 3 minutes.
8. Cold acetone (500 µl) was added and removed by centrifugation at 1,000 x *g* for 3 minutes.
9. Step 8 was repeated.
10. Cold EtOH solution (70%, 500 µl) was added and removed by centrifugation at 1,000 x *g* for 3 minutes.
11. Lysis Buffer (contains 1% protease inhibitor, 100 µl) was added and the cell pellet was dissolved by sonication.
12. Reaction Buffer A (4 µl) was added to Protein-SHifter Plus and was mixed by pipetting.
13. The solution (2 µl) at Step 11 was added to the tube at Step 12 and was mixed by pipetting.
14. Reaction Buffer B (4 µl) was added to the tube at Step 13 and was mixed by pipetting.
15. The tube at the Step 14 was incubated at 37 °C for 30 minutes.
16. 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 at Step 15 and was mixed by pipetting.
17. The solution at Step 16 was used for electrophoresis.
18. The gel was exposed with UV rays using a transilluminator for 10 minutes.
19. Separated proteins in the gel were electrophoretically transferred onto a PVDF membrane.
20. The GAPDH on the membrane was detected by the Western blot method using anti-GAPDH antibody.

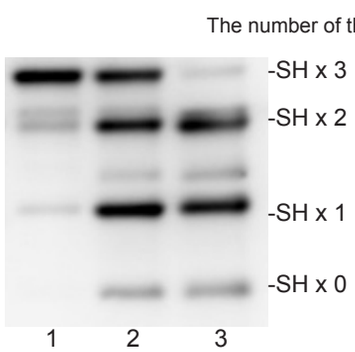

Figure 4 Detection of the number of thiol residues on the GAPDH in HeLa cell Lysate
Lane 1: no-oxidants, Lane 2: Diamide oxidation, Lane 3: H_2O_2 oxidation
- Primary antibody: Rabbit anti-GAPDH antibody, Secondary antibody:Goat anti-Rabbit antibody-POD conjugated
- 15% SDS-polyacrylamidegel, chemiluminescence detection

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" *Biochimical et Biophysical 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*". *Biochimical and Biophysical Research Communications.* **2014**, In Press.

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

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