AE-1390 *SDS-eliminant*

1. What is *SDS-eliminant* ?

AE-1390 ***SDS-eliminant*** is the reagent for removing SDS (Sodium dodecyl sulfate) from samples including SDS by 2 steps. At first, Solution A removes dodecyl sulfate ion to precipitate it by insoluble pellet. The SDS also bound to proteins directly, however, could not be removed by Solution A. Second, the non-ionic surfactant including Solution B makes mixed micelle with SDS. This step additionally removes the SDS direct-binding to the proteins. ***SDS-eliminant*** is extremely useful method in sample isolation, fractionation and purification.

● Purpose

- O Removal of SDS from the sample solution
- O Removal of SDS from the proteins isolated SDS-PAGE
- O Removal of SDS from the cell- or tissue-extracts
- O Preparation of Mass Spectrometry, electrophoresis and chromatography
- O Re-activation of enzymes* denatured by SDS * not all enzymes

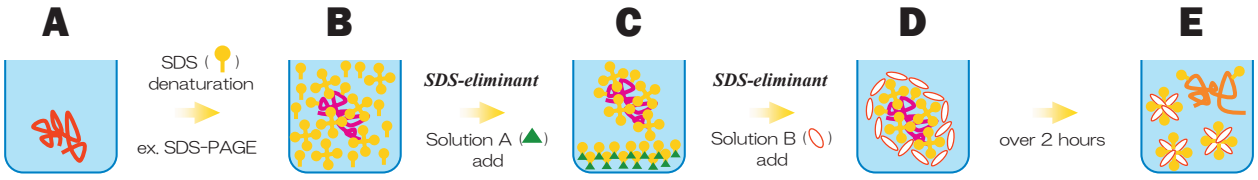
■ Feature

- ☐ Ready to Use
- ☐ Over 95% of the ionized SDS is removed briefly
- ☐ The recovery of proteins is high
- ☐ Available for the samples are small in amount (less than 1mL)

2. Specification

	Solution A	Solution B
Contents	2mL solution / a tube	2mL solution / a tube
Major constituent	KCl, Tris	non-ionic surfactant, Tris
Max sample volume	40 mL	10 mL
Conservation	Cold storage in light-blocking bag, one year guarantee	

3. Principle



A. Sample solution before SDS denaturation

··· The protein is not denaturated, the construction or activation is kept.

B. SDS denaturation of proteins

··· The protein (represented as pink figure) is denaturated*1 by SDS (represented as yellow figure, especially yellow clover-shaped shows SDS-micelle), exists in sample solution by two types ; dodecyl-sulfate ion or direct-bindng type.

C. Precipitation of dodecyl-sulfate ion

··· Add ***SDS-eliminant*** solution A to Sample solution and mixing gently. Incubate for half an hour at 4 degree C. The potassium ion including solution A (representd as green triangle) binds to dodecyl-sulfate ion and make insoluble pellet. After centrifugation, the supernatant is collected by new tube. The direct-binding SDS, however, is not removed by Solution A.

D. Addition of non-ionic surfactant

··· Add ***SDS-eliminant*** solution B to collected supernatant and mixing gently. Incubate over 2 hours at 4 degree C. The non-ionic surfactant (represented as red oval) interacts with the direct-binding SDS.

E. Mixed micellization

··· The micelles of non-ionic surfactant incorporate the direct-binding SDS and these forms of mixed micelle. The protein is released and re-activated*2 from SDS denaturation.

*1 **The denaturations of SDS are different from each protein.**

*2 ***SDS-eliminant* can remove the SDS from samples by two steps, but not absolutely.**

4. Technical data

I. SDS-rejection ratio & Protein recovery

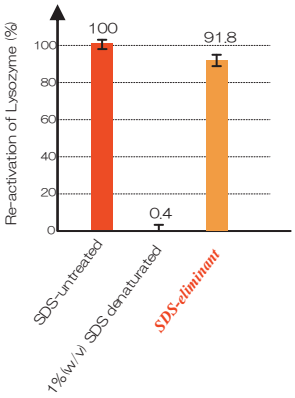
	BSA	ovalbumin	Casein	Lysozyme	Recombinant Malate Dehydrogenase
M. W.	69.2 kDa	42.9 kDa	25.1 kDa	14.3 kDa	40.0 kDa
Ratio of SDS-rejection	100 %	100 %	99 %	100 %	95 %
Protein recovery	97 %	100 %	100 %	89.5 %	No Data

* The ratio of SDS-rejection was calculated by the SDS concentration, which measured by the standard curve of Stains-ALL. The plate reader was detected the 450nm absorbance (A_{450}) of Stains-ALL which was changed by the SDS amount. It was only the method to measure the amount of dodecyl sulfate ion, not to measure the SDS direct-binding to proteins.
* Protein recovery was calculated by the Protein concentration, which measured by Pierce® BCA Protein Assay.

II. Re-activation of enzymes

A. Lysozyme

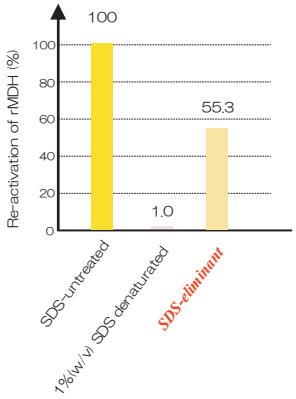
Lysozyme was 92% re-activated comparing to SDS-untreated



These ratio was calculated the 450nm absorbance (A_{450}) in bacteriolysis of *Micrococcus lysodeikticus*. The graph bars show the ratio of Lysozyme Re-activation after 60 second of sample adding. These ratios was compared to SDS-untreated sample as 100%.

B. Recombinant Malate Dehydrogenase (rMDH)

rMDH was 55.3% re-activated comparing to SDS-untreated

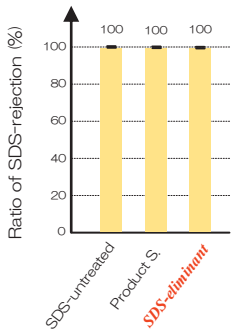


rMDH activity was detected by the 340nm absorbance (A_{340}), which was decreased by changing beta-NADH to NAD+. The graph bars show the ratio of rMDH Re-activation from 30 to 90 seconds of enzyme adding. These ratios was compared to SDS-untreated sample as 100%.

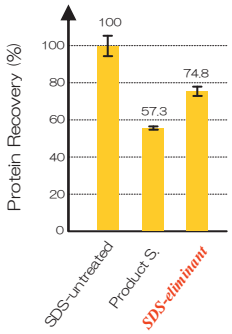
III. Competitor's Products

* The product S. (supplier is T.S. company) was compared with AE-1390 ***SDS-eliminant*** by 3 points: The ratio of SDS-rejection, Protein Recovery and Re-activation.
* AE-1390 ***SDS-eliminant*** showed superior Protein Recovery and Re-activation to the product S.

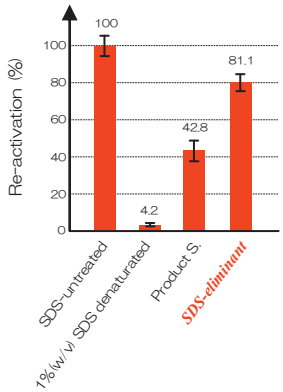
a. Ratio of SDS-rejection



b. Protein Recovery



c. Re-activation



Each analysis was conducted by the 1% (w/v) SDS-denaturated lysozyme (1mg/mL conc.). Each graph shows the ratios which was compared to the SDS-untreated samples as 100%. The ratio of SDS-rejection was calculated by Stains-ALL. Protein recovery was calculated by Pierce® BCA Protein Assay. Re-activation was calculated by the bacteriolysis of *Micrococcus lysodeikticus*.

ATTO Corporation

1-5-32 Yushima, Bunkyo-ku, Tokyo 113-0034, Japan

Tel. +81-3-3814-4824 Fax. +81-3-5684-6646

URL http://www.atto.co.jp Email eig@atto.co.jp