

Description

Non detergent sulfobetaine (NDSB) compounds are a family of non denaturing protein solubilizing agents with a wide range of applications in protein biochemistry including extraction, solubilization, and crystallization. NDSB compounds are particularly suited for protein work as they are non denaturing (most enzymes remain active in the presence of NDSB), they are zwitterionic, they possess a good solubility in water, they do not alter significantly the pH or viscosity of biological buffers, and they can easily be removed by dialysis since they do not form micelles. These agents can prevent non-specific interactions of proteins. They will however not disrupt strongly aggregated proteins.

NDSB compounds contain a hydrophilic sulfobetaine group and a short hydrophobic group. Hence, NDSB cannot form micelles and is not considered a detergent. Despite this, NDSB compounds have been used successfully to increase the yields of membrane, nuclear, and cytoskeletal-associated proteins. It has been suggested that the NDSB short hydrophobic group interacts with the hydrophobic regions on proteins to prevent aggregation. NDSB can be substituted for Sodium chloride which is often required during the isolation of halophilic proteins.⁷

NDSB-256-4T prevents protein aggregation and facilitates protein folding by interacting with the early folding intermediates. Reported to improve the *in vitro* renaturation efficiency of reduced hen egg lysozyme (60% enzymatic activity at 600 mM (NDSB-256-4T) and chemically unfolded tryptophan synthase β_2 subunit (100% enzymatic activity at 1.0 M (NDSB-256-4T)).⁸

NDSB can be a useful additive for crystal growth. This was demonstrated with lysozyme grown at pH 4.6 using Ammonium sulfate as a precipitant. Typically, Ammonium sulfate cannot be used to grow lysozyme crystals at an acidic pH. Lysozyme was also crystallized using NDSB and Sodium chloride.⁶ NDSB-195 increased the protein solubility as a function of Sodium chloride concentration (at 20°C and pH 4.6). At a concentration of 0.25 M NDSB-195 the solubility of lysozyme almost doubles, whereas at 0.75 M NDSB-195 the solubility is nearly tripled. The crystal growth rate of lysozyme in the presence of NDSB-195 is also increased. Wilson plots allowed a quantitative comparison of the quality of the crystals grown with or without NDSB-195. The curves were basically identical, indicating that crystals grown in the presence of NDSB-195 are at least as good as the controls.

Other examples of success using NDSB include:

- Desulfovibrio Gigas type II ferredoxin. Here NDSB contributed to reduction in crystal twinning and resulted in the growth of a new crystal form.⁶
- Malate dehydrogenase (MDH) : NDSB-195 has been successfully used for growing crystals of MDH. In the presence of NDSB-195, the crystal size increased from 0.1 to 0.4 mm.

Suggestions for Using NDSB

NDSB should be added before the precipitant.

If a previously successful crystallization or precipitation does not occur after addition of NDSB do not be alarmed as NDSB compounds are solubilizing agents. Gradually increase the concentration of precipitant until crystals (or precipitate) appear.

NDSB compounds are highly soluble in water, typically greater than 2.0 M.

It is a good idea to sterile filter (0.22 micron) a formulated NDSB solution into a sterile container to prevent contamination. This also avoids a slow degradation of NDSB compounds in solution that can take place in a matter of several weeks at room temperature.

NDSB used at high concentrations (0.5-1.0 M should not perturb the pH of properly buffered solutions. However, some pH drift is possible in poorly buffered systems (e.g. 10 mM Tris-HCl pH 7), so ensure at least 25 mM of buffer is present and that pH is within 0.5 pH unit of buffer pK.

References Using NDSB Compounds

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2. Biochem J 1995 Jan 1; 305 (Pt 1):337-43.
3. Electrophoresis 1995 Mar; 16(3):295-7.
4. Fold Des 1996; 1(1):21-7.
5. Eur J Biochem 1998 Aug 15;256(1):128-35.
6. J.Cryst Growth (1996) vol 168 pp 150-154.
7. Anal. Biochem. (1995) 230, 290.
8. Expert-Bezancon, N., et. al., Biophys Chem, 100, 469 (2003).

NDSB Compounds Available from Hampton Research

HR2-703	NDSB-195, 5 gram
HR2-701	NDSB-201, 5 gram
HR2-793	NDSB-211, 5 gram
HR2-791	NDSB-221, 5 gram
HR2-705	NDSB-256, 5 gram
HR2-794	NDSB-256-4T, 5 gram

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