Protocol for reduction and alkylation

INVITROGEN System – NuPAGE LDS Buffer

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Material

- Protein sample (free of sulphur-containing reducing agents!)

- NuPAGE LDS Sample Buffer (4x, pH 8.5, containing 106mM Tris HCl, 141 mM Tris Base, 2% LDS, 10% Glycerol, 0,51mM EDTA, 0,22mM SERVA Blue G250, 0,175mM Phenol Red)

- Iodoacetamide (IAA), 1M stock, (e.g. Sigma,SigmaUltra I114)

R25	Toxic if swallowed
R42/43	May cause sensitization by inhalation and skin contact
S22	Do not breathe dust
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)

- NuPAGE Reducing Agent containing 500mM DTT (Dithiotreitol),

R22	Harmful if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36	Wear suitable protective clothing

- Iodoacetamide solution should be prepared fresh. Do not use iodoacetamide if yellowish.

<u>Disposal of Iodoacetamide stock solution</u>

There is an extra waste-bottle under the big hood labeled with "*Iodoacetamide containing waste*". Before disposal the Iodoacetamide should react with DTT (1M DTT react with 2M IAA).

IAA: MW=184,96 g/mol ⇒ 1M: 1mg IAA + 5,4µl H_2O

Protocol

1. add NuPAGE LDS Sample Buffer (1X) to your dried protein sample
2. add NuPage Reducing Agent to a final concentration of ~50mM for reduction of disulfide bonds
3. denature proteins for 10 min at 70°C
4. cool sample down to RT
5. add IAA to a final concentration of ~ 120 mM
6. incubate for 20min <u>in the dark</u>
7. add Reducing Agent to a final concentration of ~ 40 mM
8. Incubate for 20min <u>in the dark</u>

There has to be an excess of IAA over DTT, since each molecule DTT can react with two molecules of IAA. Check if your protein sample contains additional DTT or other SH-molecules and increase concentration of IAA if necessary.

Example

1. add 9µl NuPAGE LDS Sample Buffer (1X) to your dried protein sample; if the sample is in solution you can add 2.5µl concentrated Buffer (4X) and water to a final volume of 9µl
2. add 1µl (500mM DTT) NuPage Reducing Agent (⇒0.0005mmol/10µl ⇒50mM)
3. denature proteins for 10 min at 70°C
4. cool sample down to RT
5. add 1.2µl 1M IAA (⇒0.0012mmol/11,2µl ⇒107mM)
6. incubate for 20min <u>in the dark</u>
7. add 1µl (500mM DTT) Reducing Agent (⇒0.0005mmol/12,2µl ⇒41mM)
8. incubate for 20min <u>in the dark</u>