Protocol for reduction and alkylation

BioRad System - Lämmli buffer

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Material

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

- Lämmli buffer, e.g. Bio-Rad, 161-0737 (e.g. 62.5mM Tris-HCl, pH6.8, 25% glycerol, 2%SDS, 0.01% Bromophenol Blue)

- 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)

- DTT (Dithiotreitol), 1M stock, (e.g. FLUKA, BioUltra 43815)

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 and DTT solutions should be prepared fresh. Do not use iodoacetamide if yellowish.

Disposal of Iodoacetamide stock solution

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 reacts with 2M IAA).

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

DTT: MW=154,25 g/mol ⇒ 1M: 1mg DTT + 6,48µl H_2O

Protocol

1. add Lämmli buffer to your protein sample
2. add DTT for reduction of disulfide bonds (final concentration: ~ 50 mM)

Sometimes Lämmli buffer already contains DTT. Then step 2 is not necessary!

3. denature proteins for 10 min at 95°C
4. cool sample down to RT
5. add IAA (final concentration: ~ 120 mM)

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.

6. incubate for 20 min in the dark
7. add DTT (~ 40 mM) to quench excess IAA and incubate for 20 min in the dark

It is important that the pH is between 7 and 8 during alkylation! Otherwise the alkylation reaction will be incomplete and Cys-containing peptides will be lost. If the colour of Bromphenolblau changes from blue to green, the solution is too acidic

Example

1. Protein sample in 20µl Lämmli buffer
2. + 1µl 1M DTT (⇒ 0.001mmol /21µl ⇒47mM)
3. denature proteins for 10 min at 95°C
4. cool sample down to RT
5. add 2.5µl 1M IAA (⇒ 0.0025 mmol/23,5µl ⇒ 106mM)
6. incubate for 20 min in the dark
7. add 1µl 1M DTT (⇒ 0.001 mmol/24,5µl ⇒41mM)
8. incubate for 20 min in the dark