

Protocol for reduction and alkylation

BioRad System - Lämmli buffer

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 \Rightarrow 1M: 1mg IAA + 5,4 μ l H₂O

DTT: MW=154,25 g/mol \Rightarrow 1M: 1mg DTT + 6,48 μ l H₂O

Protocol

1. add Lämmli buffer to your protein sample
2. add DTT for reduction of disulfide bonds (final concentration: \sim 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: \sim 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 (\sim 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 20 μ l Lämmli buffer
2. + 1 μ l 1M DTT (\Rightarrow 0.001mmol /21 μ l \Rightarrow 47mM)
3. denature proteins for 10 min at 95°C
4. cool sample down to RT
5. add 2.5 μ l 1M IAA (\Rightarrow 0.0025 mmol/23,5 μ l \Rightarrow 106mM)
6. incubate for 20 min in the dark
7. add 1 μ l 1M DTT (\Rightarrow 0.001 mmol/24,5 μ l \Rightarrow 41mM)
8. incubate for 20 min in the dark