

CERTIFICATE OF ANALYSIS

Protein Loading Buffer Pack

#R0891 20 ml 5X Protein Loading Buffer
 4 x 1.5 ml 20X Reducing Agent

Lot:

Store: 5X Protein Loading Buffer at -20°C or at 4°C
 20X Reducing Agent at -20°C

In total 5 vials.

Description

The Protein Loading Buffer Pack is used for the preparation of protein samples for SDS-PAGE (1). The buffer contains all the necessary reagents for complete disruption of high-order protein structures. The SDS included in the buffer binds to hydrophobic regions of the protein, causing the protein to unfold and giving it a negative charge. The reducing agent (DTT) breaks disulfide bonds and destroys residual secondary structures. As DTT is prone to oxidation during multiple freeze-thaw cycles it is supplied in separate vials.

Composition

5X Loading Buffer: 0.313 M Tris HCl (pH 6.8 at 25°C), 10% (w/v) SDS, 0.05% (w/v) bromophenol blue, 50% (v/v) glycerol.

20X Reducing Agent: 2 M DTT.

QUALITY CONTROL

Tested in 12% SDS-PAGE with three different proteins.

Quality authorized by:  Jurgita Zilinskiene

Recommendations for sample preparation

Step	Procedure
Thawing	Thaw the Reducing Agent at room temperature. Dissolve precipitated solids in the Loading buffer (if any) at 37°C.
Mixing	<ol style="list-style-type: none">1. Vortex gently the components of the pack to ensure that the solutions are homogeneous.2. Place 2 µl of 20X Reducing Agent into clean microcentrifuge tube*.3. Add 10 µl of 5X Protein Loading Buffer.4. Add protein sample solution.5. Add Water, nuclease-free (#R0581) to 50 µl.
Denaturing	Heat samples at 100°C for 3-5 minutes.
Loading	Centrifuge briefly and apply directly to a SDS-polyacrylamide gel.

*For silver staining *see* Note.

Notes

- For silver staining DTT concentration in the sample should not exceed 50 mM. Higher DTT concentration in protein sample may cause streaking or yellowing of the gel. If initial protein sample contains >50 mM DTT, do not add Reducing Agent into the protein loading buffer.
- Because of SDS in a loading buffer the pack should not be used for native polyacrylamide gel electrophoresis.

SAFETY INFORMATION



20X Reducing Agent

Xn Harmful

Hazard-determining component of labeling:

(R*,R*)-1,4-dimercaptobutane-2,3-diol

Risk phrases

R22 Harmful if swallowed.
R3638 Irritating to eyes, respiratory system and skin.

Safety phrases

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.

Reference

1. Laemmli, U.K., Nature, 227, 680-685, 1970.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.