2X Protein Loading Buffer (Reducing)

Introduction

2X Protein Loading Buffer (Reducing) is an improved 5-fold concentrated protein loading buffer. Its main components include SDS, a sulfhydryl reducing agent, bromophenol blue, and buffer salts. SDS binds to proteins, imparting a large net negative charge to the protein-SDS complex, thereby masking the intrinsic charge differences among various proteins. SDS also disrupts intra- and inter-molecular hydrogen bonds, destroying the secondary and tertiary structures of protein molecules. The sulfhydryl reducing agent breaks disulfide bonds between cysteine residues, disrupting the quaternary structure of proteins and eliminating structural differences between proteins, so that the electrophoresis migration rate depends only on molecular weight. Bromophenol blue serves as a tracking dye during electrophoresis, indicating approximately when the run should be terminated.

2X Protein Loading Buffer (Reducing) is suitable for loading protein samples in conventional SDS-PAGE.

Protocol

1. Thaw 2X Protein Loading Buffer (Reducing) at room temperature or in a water bath not exceeding 37°C.
2. Use the buffer at a ratio of 1 volume of 2X loading buffer to 1 volume of protein sample.
3. Mix thoroughly. Heat at 95°C in a water bath or heating block for 5–10 minutes to fully denature the proteins.
4. Cool to room temperature, then centrifuge at 10,000–14,000 rpm for 2–5 minutes. Load the supernatant into the wells of an SDS-PAGE gel.
5. Electrophoresis is typically stopped when the blue dye front reaches the bottom of the gel.

Note

1. Ensure that 2X Protein Loading Buffer (Reducing) is completely dissolved before use.
2. When using this product for protein denaturation, excessively high temperatures (e.g., 100°C) or prolonged heating (e.g., more than 15 minutes) may cause protein degradation or abnormal dye coloration.
3. The position of the bromophenol blue dye front varies with gel concentration:
   - At 8% polyacrylamide gel, the dye front corresponds to approximately 30 kDa.
   - At 12% gel, the dye front corresponds to approximately 20 kDa.
   - At 15% gel, the dye front corresponds to approximately 10 kDa.

Determine the stopping time based on your target protein size.

4. For your safety and health, please wear a lab coat and disposable gloves when handling this product.
5. Shipping and Storage: Shipped with blue ice. Store at -20°C; stable for more than one year.
6. This product is for scientific use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com