50X Reducing Buffer

Introduction

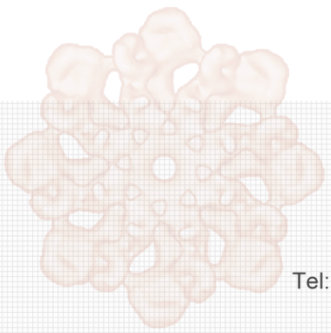
50X Reducing Buffer is a modified 50-fold concentrated sample reducing agent. It contains the reducing agent DTT. 50X Reducing Buffer can be used in combination with 5X Protein Loading Buffer (No Reducing Buffer) (Catalog No. K1165) or 2X Protein Loading Buffer (No Reducing Buffer) (Catalog No. K1167). It is intended for loading protein samples in routine SDS-PAGE.

Protocol

1. Thaw/dissolve the 50X Reducing Buffer at room temperature or in a water bath not exceeding 37°C.
2. Use the 50X Reducing Buffer as follows: 0.16 ml of 50X Reducing Buffer for 5 ml of 2X Protein Loading Buffer (No Reducing Buffer), 0.32 ml of 50X Reducing Buffer for 10 ml of 2X Protein Loading Buffer (No Reducing Buffer), 0.4 ml of 50X Reducing Buffer for 5 ml of 5X Protein Loading Buffer (No Reducing Buffer), 0.8 ml of 50X Reducing Buffer for 10 ml of 5X Protein Loading Buffer (No Reducing Buffer).
3. Then, mix the protein sample with the prepared loading buffer at the following ratio: add 1 volume of 2X Protein Loading Buffer (with Reducing Buffer) to 1 volume of protein sample, or add 1 volume of 5X Protein Loading Buffer (with Reducing Buffer) to 4 volumes of protein sample.
4. Mix thoroughly. Heat in a 95°C water bath or heating block for 5-10 minutes to fully denature the protein.
5. Cool to room temperature, then centrifuge at 10,000-14,000 rpm for 2-5 minutes. Load the supernatant into the wells of the SDS-PAGE gel.
6. Typically, stop electrophoresis when the blue dye front reaches near the bottom of the gel.

Note

1. The 50X Reducing Buffer must be completely dissolved before use.
2. When using polyacrylamide gels, the bromophenol blue dye front migrates at approximately 30 kDa for 8% gels, approximately 20 kDa for 12% gels, and approximately 10 kDa for 15% gels. Please determine the stopping time of electrophoresis based on your target protein size.
3. Storage and transport: Store at -20°C for up to 1 year. Transport with blue ice packs.
4. This product is for scientific use only.



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