

## 5× SDS-PAGE Protein Loading Buffer – Instruction Manual

### Catalogue Information

Catalogue No.	Product Name	Pack Size
BL530B	5× SDS-PAGE Protein Loading Buffer, 15 mL (Odourless)	15 mL

### Product Description

The 5× SDS-PAGE Sample Loading Buffer (Odorless) is mainly composed of Tris, SDS, tracking dye (bromophenol blue), and reducing agent. SDS binds to proteins, giving them a large negative charge, which masks the intrinsic charges of proteins and eliminates charge differences among proteins. SDS also disrupts intra- and inter-molecular hydrogen bonds, weakening secondary and tertiary structures. The reducing agent breaks disulfide bonds between cysteine residues, eliminating structural variations. In the end, proteins (subunits) differing only in size migrate based primarily on molecular weight. Bromophenol blue serves as a tracking dye to roughly indicate when electrophoresis is complete.

This odorless 5× buffer is a modified version that uses an odorless, more stable, water-soluble reducing agent, TCEP (tris(2-carboxyethyl)phosphine hydrochloride), instead of the strong-smelling DTT or  $\beta$ -mercaptoethanol. This ensures the buffer is safer, more pleasant to work with, especially during heating or routine usage.

### Instructions for Use

1. Dissolve the 5× buffer (containing TCEP) in a water bath at room temperature or no more than 37 °C. Once dissolved, store at room temperature. Avoid prolonged exposure to the water bath. After use, store at -20 °C.
2. Mix your protein sample and 5× Sample Loading Buffer in a 4:1 ratio (i.e. 5× buffer diluted to 1×), ensuring thorough mixing. For example, add 10  $\mu$ L of 5× buffer to 40  $\mu$ L protein sample. If the protein concentration is too high, dilute with double-distilled water.
3. Heat at 95 °C for 5–10 minutes to fully denature proteins.
4. Cool to room temperature; centrifuge at 10,000–14,000 g for 2–5 minutes; use the supernatant for loading onto the gel.
5. Run electrophoresis until the tracking dye (blue) is near the bottom of the gel, then stop.

### Notes

1. For protein denaturation, heating at 95 °C for 5 minutes is recommended. Excessively high temperatures (e.g. 100 °C) or prolonged heating (over 15 minutes) may cause protein degradation or abnormal color of the loading buffer's tracking dye.
2. This buffer (5X, containing TCEP) is free from toxic  $\beta$ -mercaptoethanol and odorous DTT, while providing equivalent reducing power and electrophoresis performance.
3. 5× buffer (with TCEP) must be fully dissolved before use. It is advised to aliquot and freeze based on usage needs to avoid repeated freeze-thaw cycles.
4. This product is for research use only by professionals. Not for clinical diagnostic, therapeutic, food, or drug use.
5. For safety and health, wear lab coat and disposable gloves when using.

### Storage and Stability

1. Store at -20 °C.
2. Valid for 1 year when stored under recommended conditions.



**Notice**

For in vitro research use only, not for diagnostic or therapeutic use. This product is not a medical device.

Contact:

- [Support@darwinstech.com](mailto:Support@darwinstech.com)