

Store at RT
and -20°C
#7723

Red Loading Buffer Pack



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For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Item #	Kit Quantity	Storage Temp
3X Red Loading Buffer	67068	8 ml	RT
30X Reducing Agent (1.25 M DTT)	14265	1 ml	-20°C

Description: The Red Loading Buffer Pack is used to lyse cells for western blotting analysis.

Solutions and Reagents:

3X Red Loading Buffer (#67068): 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% (w/v) SDS, 30% glycerol and 0.03% (w/v) phenol red. (Store at room temperature.)

30X Reducing Agent (#14265): 1.25 M dithiothreitol (DTT) (Store at -20°C.)

Directions for Use:

1. Prepare fresh 3X Reducing SDS Loading Buffer by adding 1/10 volume 30X DTT Reducing Agent (#14265) volume of 3X Red Loading Buffer (#67068).
2. 3X Red Loading Buffer (#67068) to a 1X solution using ddH₂O. This product supplies enough 3X material to make 24ml of 1X solution.
3. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
4. Lyse cells by adding 1X SDS Loading Buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm² plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
5. Sonicate for 10-15 seconds to shear DNA and reduce sample viscosity.
6. Heat a 20 µl sample to 95-100°C for 5 minutes; cool on ice.
7. Microcentrifuge for 2-5 minutes.
8. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).