

Store at RT  
and -20°C  
**#7722**

# Blue Loading Buffer Pack



**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

rev. 06/07/18

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

Product Includes	Item #	Kit Quantity	Storage Temp
3X Blue Loading Buffer	56036	8 ml	RT
30X Reducing Agent (1.25 M DTT)	14265	1 ml	-20°C

**Description:** The Blue Loading Buffer Pack is used to lyse cells for Western blotting analysis

**Solutions and Reagents:**

3X Blue Loading Buffer (#56036): 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% (w/v) SDS, 30% glycerol and 0.03% (w/v) bromophenol blue. (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 Blue Loading Buffer (#56036).
2. Dilute 3X Blue Loading Buffer (#56036) to a 1X solution using ddH<sub>2</sub>O. 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 cm<sup>2</sup> 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).