

5X Lysis Binding Buffer

Catalog No: 52110

Format: 20 ml

Quality Control: The 5X Lysis Binding Buffer (part number 106031) can be used, along with the enclosed Protease Inhibitor Cocktail (part number 106048) to prepare whole-cell extracts for use in either the Ras GTPase Chemi ELISA Kit (52097) or in the KRAS In-well Lysis ELISA Kit (52100/52105). Please see their respective manuals for guidance on how much lysate to use for each assay.

To prepare whole-cell extracts, the following protocol can be used for a 100 mm culture dish or 2×10^7 cells.

1. For each 100 mm culture dish or 2×10^7 cells, prepare 500 μ l 1X Complete Lysis Binding Buffer by mixing 5 μ l Protease Inhibitor Cocktail, 100 μ l 5X Lysis Binding Buffer, and 395 μ l distilled water.

NOTE: Protease inhibitors lose their activity after 24 hours once diluted, so make the 1X Complete Lysis Binding Buffer the day of use. The remaining amount should be discarded if not used the same day.

2. Remove culture media from cells and wash with 5 ml ice-cold PBS.

3. For adherent cells add 500 μ l of 1X Complete Lysis Binding Buffer to the plate and scrape cells with a rubber policeman. For pelleted cells resuspend cell pellet in 500 μ l 1X Complete Lysis Binding Buffer.

4. Transfer suspended cells to a microcentrifuge tube. Incubate 15 minutes on ice.

5. Vortex tube for 10 seconds and then centrifuge for 10 minutes at 14,000 rpm at 4°C.

6. Collect the supernatant at 4°C.

7. Measure the protein content using a Bradford-based assay.

8. Extracts can be aliquoted and frozen at -80°C for later use. However, for best results, use immediately. Dilute whole-cell extracts in 1X Complete Lysis Binding Buffer to desired concentration and transfer 50 μ l to each ELISA well.

Storage and Guarantee: The 5X Lysis Binding Buffer and accompanying Protease Inhibitor Cocktail are shipped on dry ice. Upon receipt, store the 5X Lysis Binding Buffer at 4°C and the Protease Inhibitor Cocktail at -20°C. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.