

ClearBand Lyse & Load

Cat#: orb1109249 (Manual)

General Information

Lyse&Load is a premixed buffer, which combines RIPA Lysis Buffer and Laemmli Sample Buffer in one solution for protein sample preparation prior to loading on SDS-PAGE. 25 µl B-mercaptoethanol should be added per 1 ml Lyse&Load prior to use.

Protocol

1. Add 25 µl B-mercaptoethanol per 1 ml Lyse&Load prior to use.
Note that Lyse&Load does not include protease or phosphatase inhibitors. If desired, protease and phosphatase inhibitors can be added to Lyse&Load just prior to use to prevent proteolysis and maintain phosphorylation status of proteins.
2. Resuspend and wash 1×10^6 cells in chilled PBS and discard the supernatant.
3. Lyse cell pellet in 0.1 ml Lyse&Load and vortex thoroughly. Incubate 30 sec at room temperature. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst.
4. Homogenize the lysate using a syringe and needle by passing the lysate through a 20-gauge (0.9 mm) needle, attached to a sterile plastic syringe, at least 5–10 times or until a homogeneous lysate is achieved.
5. Keep samples in ice for 5 minutes to dissolve structures and complexes.
Note that centrifugation at 4 C°, 13.000 rpm for 10 minutes may result in development of better western blot bands depending on the experimental set up or cell type. If samples are centrifuged, take the supernatant in a new Eppendorf tube and continue with step 6.
6. Heat for 5 minutes in boiling water.
7. Load boiled samples into the wells of SDS-PAGE