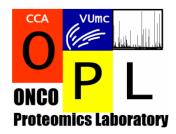
Preparation of Cell Line Lysates



IMPORTANT GENERAL REMARKS:

- ✓ Lysates are made directly in PAGE sample buffer (1xRSB); this is more efficient than solubilisation in, e.g., RIPA buffer.
- ✓ To avoid proteolysis, work fast during the suspension of cells in sample buffer, and heat as soon as possible.

Method

Making Lysates

- 1. Wash cells 3x with PBS or serum-free medium:
 - monolayers: wash cell layer in the dish.
 - suspension cells: wash cell pellet.
- 2. Quickly take up cells into 1xRSB:
 - monolayers: scrape cells directly into 1xRSB (50-100 μl/10⁶ cells).
 - suspension cells: resuspend cell pellet in 1xRSB (1 ml/T75 flask). If the lysate is really slimy, add another 0.5 ml 1xRSB.
- 3. As soon as possible, heat suspensions for 5-10 min at 99 °C.
- 4. Lysates can be stored at -20 °C (short term) or -80 °C (long term).

Testing

- 5. Run a test PAGE gel with different loadings of the lysate (e.g., 10 μl, 20 μl, 30 μl).
- 6. Fix gel and continue with Coomassie Blue staining & destaining (see appropriate protocol).
- 7. Select an optimal loading volume based on the gel patterns: the aim is to load the maximum without protein smearing along the lane (sign of overloading).

Solutions

- o Phosphate-Buffered Saline (PBS)
- standard ordered bottle.
- o **1xReducing Sample Buffer (1xRSB):** (depending on which gel system you are aiming for)
- 1x reducing SDS-PAGE sample buffer for self-cast (Laemmli) gels, or
- 1x reducing NuPAGE sample buffer for pre-cast NuPAGE gradient gels.
- o Fixing Solution:
- 50% (v/v) ethanol
- 3% (w/v) phosphoric acid (H₃PO₄)

Materials & Equipment

thermomixer (Eppendorf)