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/10^6 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 ^{0}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

- **Phosphate-Buffered Saline (PBS)**
  - standard ordered bottle.

- **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.

- **Fixing Solution:**
  - 50% (v/v) ethanol
  - 3% (w/v) phosphoric acid (H_3PO_4)

Materials & Equipment

- thermomixer (Eppendorf)