Preparation of Lysates from Cultured Cells

Step 1. Prepare adherent cultured cells

1. Culture 1×10^6 to 1×10^7 cells.
2. Trypsinize the cells and remove the cells using a cell scraper.
3. Transfer cells to a 15 mL conical tube and spin at 500 × *g* at 4°C for 5 mins.
4. Carefully aspirate the supernatant and resuspend the cells in 5 mL of ice-cold PBS and spin again at 500 × *g* at 4°C. Repeat this step once with 5 mL of ice-cold PBS.
5. Remove as much supernatant as possible without disturbing the cell pellet. Proceed to Step 3 Lyse Cells.

Step 2. Prepare suspension cells

1. Culture 1×10^6 to 1×10^7 cells.
2. Transfer cells to a 15 mL conical tube and spin at 500 × *g* at 4°C for 5 mins.
3. Carefully aspirate the supernatant and resuspend the cells in 5 mL of ice-cold PBS and spin again at 500 × *g* at 4°C.
4. Remove as much supernatant as possible without disturbing the cell pellet. Proceed to Step 3 Lyse Cells.

Step 3. Lyse cells

1. To the prepared cultured cells from Step1 or Step 2, add 200 µL of ice-cold ProcartaPlex™ Cell Lysis Buffer (EPX-99999-000) to the cell pellet.
2. Pipette up and down 8-10 times and incubate on ice for 5 mins.
3. Transfer the entire contents to a 1.5 mL microcentrifuge tube.
4. Centrifuge at 14,000 × g (benchtop centrifuge) for 10 mins at 4°C.
5. Transfer the supernatant to a new tube. Assay the samples immediately or store them at -80°C.

Optional

Perform a protein assay using a small aliquot of the lysate sample by following the manufacturer's protocol of a protein assay kit. We recommend Lowry Protein Assay for measuring protein concentrations of cell lysates. We recommend adjusting all samples to equal protein concentration at a range of 4-8 mg protein/mL using ice-cold ProcartaPlex™ Cell Lysis Buffer.

To proceed with the ProcartaPlex Protocol, add 25 µL of Universal Assay Buffer (EPX-11111-000) to 25 µL of the prepared sample to each sample well.

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use	Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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