Isolation of cell fractionation

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Abstract

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Protocol

Step 1.

Step 2.
A total of 5 × 106 cells were collected after brief trypsinization, followed by two more washes with PBS, and the cell pellet was resuspended in 200 µl of mitochondria extraction buffer containing 0.02 mM phenylmethanesulfonyl fluoride (PMSF) and proteinase inhibitors (Keygentec, Nanjing, China).

Step 3.
After incubating on ice for 20 min, the cells were homogenized using a glass Dounce and pestle.

Step 4.
The homogenates were centrifuged at 600 g for 15 min at 4℃, and the resulting supernatant was collected and centrifuged at 11,000 g for 15 min at 4℃ to separate the mitochondria (pellet) and cytoplasmic proteins (supernatant).The mitochondria pellet was lysed in mitochondria extraction buffer.