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Protocol status: Working
We use this protocol and it's working

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Mitochondria purification

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ABSTRACT

This protocol describes how to purify mitochondria from cell culture

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85959

Mitochondria purification

15m

1 HEK293T cells were trypsinized (0.05% Trypsin 00:05:00) and collected by centrifugation (500g 00:05:00). 10m

2 Cells were washed twice with NKM buffer (1 mM Tris HCl, pH7.3, 0.13 M NaCl, 5 mM KCl, and 7.5 mM MgCl2), and resuspended in six packed cell volumes of homogenization buffer (10 mM Tris pH 7.4, 10 mM KCl, and 0.15 mM MgCl2).

3 Cells were homogenized by 10 passages through a 22G needle.

4 Cell homogenates were mixed gently with the same volume of 2.3 M sucrose solution and centrifuged at 1200×g for 00:05:00 4 °C to remove unbroken cells and large cell debris. 5m

5 The recovered supernatant fractions were centrifuged at 7000×g for 00:10:00 4 °C . 10m

6 Mitochondria enriched in the pellet fraction were resuspended in three packed cell volumes of Mitochondria Suspension Buffer (10 mM Tris, pH 7.3, 0.15 mM MgCl2, and 0.25 mM sucrose).