

Isolation of mitochondria

This protocol will focus on isolation of mitochondria from rat tissues. All the following steps need to be performed at 4°C (on ice) to protect the mitochondria.

1. Tissue preparation

Carefully dissect the tissue you want to study and immediately wash it in the buffer designed for mitochondrial isolation presented in «buffers composition» section. This first step allows to remove a high amount of blood from the tissue. Using scissors, carefully mince your tissue (don't forget to stay on ice).

Isolation of mitochondria

2. Protease treatment

Only few tissues require a protease treatment. This step is crucial to isolate mitochondria from the heart and skeletal muscles. These two kinds of tissues contain a high amount of collagen which can disturb the extraction of mitochondria. The collagen network thus needs to be partly digested by protease to facilitate the extraction of mitochondria.

Expose your muscle or heart tissue to a solution containing 2 mg of a bacterial proteinase type XXIV (Sigma-Aldrich: P8038) per ml of isolation buffer during five minutes at 4°C.

Isolation of mitochondria

3. Homogenization

Place your tissue into a potter, add approximately 3 ml of isolation buffer to fill in the potter. This addition of isolation buffer is required to ensure a correct protection of your mitochondria, and especially after protease treatment (only required for muscle and heart tissues). Start the homogenization. This step can be stopped when your tissue is completely homogenized (approximately 5 minutes depending on the tissue your are studying).

Isolation of mitochondria

4. Differential centrifugation

The aim of this step is to remove residual erythrocytes and other cellular components such as nuclei, lysosome... and to only keep mitochondria.

When the homogenization is finished, place your sample in a tube designed for your centrifuge. Make sure you placed an other tube in the centrifuge, equilibrated with the tube containing your sample. The following protocol is used to isolate the mitochondria :

1. Spin your sample at 7900/8000g during 20min: this step is particularly crucial for muscle and heart to remove

Isolation of mitochondria

the protease (located in the supernatant). You also will remove some other organelles of the cells with a very low density. Your mitochondria are now located in the pellet. Resuspend this pellet using the isolation buffer, and equilibrate your tube for the next spinning

2. Spin your sample at 1000g during 20min: this step will allow you to remove erythrocytes and some broken parts of cells or extracellular matrix (especially for muscle tissues). Your mitochondria are now in the supernatant. Put this supernatant in a new tube, equilibrate with an empty one to be ready for the last step.

Isolation of mitochondria

3. Spin your sample at 7900/8000g during 20min: this step allow you to place your mitochondria in the pellet. It also allows you to remove residual low density components such as lysosomes for example.
4. Resuspend the pellet using a very low volume of the isolation buffer to obtain your final solution of mitochondria with a high concentration of mitochondrial proteins. This high concentration is required for functional assays. (400 μ l of isolation buffer can be for example used for isolation of mitochondria from skeletal muscle)

Isolation of mitochondria

So now you have your mitochondria ready for functional assays! But don't forget to determine the protein concentration of your solution of mitochondria in order to be able to normalize your results!

1. Reference

1. Cannon, B. and O. Lindberg, *Mitochondria from brown adipose tissue: isolation and properties*. Methods Enzymol, 1979. **55**: p. 65-78.