AZUL MITOCHONDRIA DNA EXTRACTION KIT

azooka

INTENDED USE

AZUL Mitochondrial DNA Extraction Kit is an easy and efficient system for the isolation of total DNA from intact mitochondria.

SUMMARY AND EXPLANATION

This kit uses a silica-based spin column technology for isolating DNA from biological samples, thereby eliminating toxic phenol-chloroform extractions. The eluted DNA is suitable for all sensitive downstream applications such as qPCR and Next-Generation sequencing.

PRODUCT FEATURES

- Rapid purification of high-quality, ready-to-use DNA.
- No organic extraction or alcohol precipitation.
- Consistent and high yields.
- Complete removal of contaminants and inhibitors for reliable results.
- Kit formats for low- to high-throughput – options for automation of all kits.

PRECAUTIONS

- Avoid all skin contact with reagents in this kit. In case of contact, wash thoroughly with water.
- AZUL Mitochondria DNA Extraction Kit is intended for use as supplied. Do not dilute or add other components to the AZUL Mitochondria DNA Extraction Kit.

DIRECTIONS FOR USE

Mitochondria Isolation from Cell Suspension / Adherent Cells:

1. Collect cells (5 x 10^7) by centrifugation at 600 rpm for 5 mins at 4°C. For adherent cells, trypsinize the cells and centrifuge at 600 rpm for 5 mins at 4°C.
2. Wash cells with 2 mL of ice-cold Stabilization Buffer. Centrifuge at 600 rpm for 5 mins at 4°C. Discard supernatant.
3. Resuspend cells in 1 mL of ice cold 1x MEB1 Buffer and incubate on ice for 10 min.
4. Homogenize cells in an ice-cold dounce tissue grinder. Perform the task with the grinder on ice. 50 - 100 passes with the grinder are recommended; however, efficient homogenization may depend on the cell type.
5. Transfer homogenate into a 1.5 mL microcentrifuge tube, and centrifuge at 700 rpm for 10 mins at 4°C. This step removes nuclei and intact cells (in pellet).
6. Transfer supernatant into a new 1.5 mL tube, and centrifuge at 10,000 rpm for 20 mins at 4°C.
7. Discard supernatant and resuspend the pellet in 1 mL 1x MEB2 Buffer and centrifuge at 10,000 rpm for 20 mins at 4°C.
8. Discard the supernatant. The pellet is the isolated mitochondria.

Mitochondria Isolation from Animal tissues:

1. Take ≥100 mg of tissue and wash briefly with 2 mL Stabilization Buffer to remove any blood traces.
2. Finely mince the tissues using a scissor and wash it with 2 mL Stabilization Buffer.
3. Decant the medium and add 3 mL of 1x MEB1 Buffer and incubate on ice for 10 min.
4. To proceed further, follow the steps from point 4 as mentioned above

Mitochondrial DNA Extraction:

1. Add 400 µL of MT Lysis Buffer to the pellet in order to lyse the mitochondria and incubate on ice for 10 mins.
2. Add 5 µL of Proteinase K and incubate at 56°C water bath for 60 mins.
3. Add 100 µL of Binding Buffer 1 to the tube and mix well (white precipitate may be observed).
4. Add 10-20 µL of RNase A, mix well and incubate at 37°C for 30 mins to remove RNA contamination.
5. Centrifuge the contents at 15,000 rpm for 10 mins at RT. Transfer the clear supernatant to a new microfuge tube.
6. To this suspension, add 500 µL of Binding Buffer 2 and mix well.
7. Transfer the lysate to a clean spin column. Centrifuge at 10,000 rpm for 2 mins at RT.
8. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until complete lysate has been transferred into the column and centrifuged.
9. Wash the spin column with 500 µL Wash Buffer (WB) at 10,000 rpm for 1 min and discard the flow through. Repeat this step again.
10. Keep the purification column in a clean, sterile 1.5 mL microfuge tube and add 30 µL of Elution Buffer or DNase/RNase-free water to the center of the column.
11. Centrifuge the column at 10,000 rpm for 2 mins.
12. Discard the purification column and store the eluted mtDNA at -20°C or -80°C until use.

KIT COMPONENTS

Components	For 50 preps	For 25 preps
Stabilization Buffer (STB)	200 mL	100 mL
2X MT Extraction Buffer	200 mL	100 mL
MT Lysis Buffer (LB)	30 mL	15 mL
Enzyme Mix	32 mL	16 mL
Binding Buffer 1 (BB1)	6 mL	3 mL
Binding Buffer 2 (BB2)	30 mL	15 mL
Wash Buffer (WB)	50 mL	25 mL
Elution Buffer(EB)	4 mL	2 mL
Proteinase K	0.3 mL	150 µL
RNase A	1 mL	0.5 mL
Spin Column	50 (Pouch pack)	25 (Pouch pack)

CAUTION

- Check the buffers for any salt precipitation before every use.
- Re-dissolve any precipitate by warming the solution to 37°C, then cool it back to room temperature before use.
- During operation, always wear a lab coat, disposable gloves, protective goggles and mask.

KIT STORAGE AND STABILITY

- Store the kit at room temperature.
- Viable for 1 year if stored at appropriate conditions.

ORDERING INFORMATION

Please call us at +91 8088747968 or mail at hello@azooka.life for any queries or assistance.
Additional information can be found online at www.azooka.life

CE IVDR ISO 13485 CERTIFIED

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