CE IVDR

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AZUL MITOCHONDRIAL DNA EXTRACTION KIT

GOOD YIELDS FOR USE IN PCR/SEQUENCING

PRODUCT BROCHURE

Cat No-DE117

ISO 13485 CERTIFIED

PRODUCT DESCRIPTION

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

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)

SPECIFICATIONS

Format	Spin column
Sample type	Animal/Plant Tissues, Suspension cells, Adherent cells
Equipment	Microcentrifuge
Processing time	Depends on incubation time
Sample amount	Tissue ≥ 100 mg Cells - 5 x 10^7
Type	Total mtDNA
Sample storage	Eluted DNA should be stored at ≤ -20°C
Yield	1-10 µg
Purity	A260/280 ≥ 1.8
Kit Storage	2-8°C
Kit Validity	Viable for 1 year if stored at appropriate conditions

- *Avoid harsh or prolonged homogenization, as this may prevent the isolation of intact Mitochondria.*
- *Use of ice-cold 1x MEB1 and 1x MEB2 Buffer is reccomended.*
- *On addition of elution buffer to the column incubate at RT for 2 mins, for efficient elution.*

BUFFER PREPARATION

- Prepare 1x MEB1 and 1x MEB2 Buffers from 2x MT-Extraction Buffer before DNA extraction.
- For 1x MEB1 Buffer - Add 75 mL MilliQ water to 75 mL of 2x MT-Extraction Buffer, 15 mL of enzyme mix and store at 2-8°C.
- For 1x MEB2 Buffer - Add 25 mL MilliQ water to 25 mL of 2x MT-Extraction Buffer, 1 mL enzyme mix, and store at 2-8°C.
- Add 150 µL of Milli Q /Nuclease Free water to Proteinase K, dissolve completely and store at -20°C.
- Add 0.5 mL of Milli Q /Nuclease Free water to RNase A, dissolve completely and store at -20°C.
- Add 16 mL of Milli Q /Nuclease Free water to Enzyme mix, dissolve completely and store at -20°C.

DNA EXTRACTION PROTOCOL

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.

NOTE:

- *Falcon tubes (15 mL) can be used for centrifugation, if 3-4 mL of 1x MEB1 buffer is used for homogenization of tissues.*
- *After resuspending the pellet in 1 mL of 1x MEB2 Buffer, the suspension can be transferred to a 1.5 mL microfuge tube for further isolation.*

Mitochondria Isolation from Plant tissues:

1. Take the plant tissue to pre-chilled mortar and pestle, grind in homogenization buffer for 30 seconds. The homogenizing medium should be added in a ratio of at least 2 mL/ gram of non-green tissue or at least 4 mL/ gram of green tissue.
2. The final suspension is filtered using 2-3 layers of muslin cloth to centrifuge tubes to remove the majority of the starch, cell debris and kept at 4°C.
3. Centrifuge for 5 min at 300 rpm at 4°C, the supernatant following centrifugation is decanted gently into another set of centrifuge tubes, take care not to transfer the pelleted material, which contains starch, nuclei, and cell debris. The supernatant is then centrifuged for 15-20 min at 12,000 rpm at 4°C, and the resulting high-speed supernatant is discarded.
4. The tan or green colored pellet in each tube is resuspended in 5-10 ml of a standard wash medium.
5. The resuspended organelles are transferred to 50-ml centrifuge tubes, and the volume in each tube is adjusted to 40 ml with wash medium and the samples are centrifuged at 1000 rpm for 5 mins.
6. The supernatants are transferred into another set of tubes and the organelles are sedimented by centrifugation at 12,000 rpm for 15-20 mins. The high-speed supernatant is discarded once again and the washed organelles are resuspended uniformly in approximately 2 ml of wash medium.
7. To proceed further, follow the steps from point 4 as mentioned above.

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DNA EXTRACTION PROTOCOL

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.

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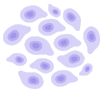
FLOW DIAGRAM OF DNA EXTRACTION PROTOCOL

Weigh ≥ 100 mg of tissue, cut into pieces



Cells (5 x 10^7)

1-3 mL 1x MEB1 Buffer

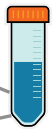
Homogenize cells in an ice-cold dounce tissue grinder

Transfer homogenate and centrifuge at 700 rpm for 10 mins

Supernatant + Centrifuge at 10,000 rpm for 20 mins

Pellet + 1 mL 1x MEB2 Buffer

Centrifuge at 10,000 rpm for 20 mins



Isolated Mitochondria

Add 400 µL of MT Lysis Buffer + 5 µL Proteinase K

Incubate at 56°C for 1 hour

Add 100 µL Binding Buffer 1, 10-20 µL RNase A

Incubate at 37°C for 30 mins

Centrifuge at 15,000 rpm for 10 mins

Supernatant + 500 µL Binding Buffer 2

Mix well
Transfer lysate to Spin column

Centrifuge at 10,000 rpm for 2 mins

Add 500 µL Wash Buffer

Centrifuge at 10,000 rpm for 1 min

Repeat this step

Add 30 µL Elution Buffer

Centrifuge at 10,000 rpm for 2 mins

Eluted mtDNA

Store at -20°C or -80°C until use

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSES	SUGGESTED SOLUTIONS
Low DNA Yield	**Sample input:** Too much input or significantly less sample used.	Use less input material or increase the volume of 1x MEB1 Buffer/Homogenization buffer and homogenize thoroughly. Use of ≥ 100 mg tissue and 5 x 10^7 cells are recommended for good DNA yield.
	Incomplete Debris Removal or incomplete lysis/homogenisation can cause cellular debris to clog or overload the column and leech salts into DNA eluate.	Increase the volume of Lysis Buffer to ensure complete lysis/ homogenisation. Be sure to centrifuge and pellet any cellular debris and transfer the supernatant while avoiding any pellet debris.
Low DNA Purity(A260/A280)	Improper sample handling results in ethanol or salt contamination.	Make sure lysate and wash buffers have passed entirely through the matrix of the column. This may require centrifuging at a higher speed or longer time.
RNA Contamination	Too much tissue/cell suspension used.	**To remove RNA:** Perform RNase A treatment post-purification, if RNase A treatment during the isolation was not sufficient to remove RNA contamination, then re-purify the treated sample.
DNA Degradation	Use of old tissue / cell suspension samples.	**To prevent DNA degradation:** Immediately collect and lyse fresh samples into 1x MEB1 Buffer.

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ORDERING INFO

CATALOG NO	PRODUCT	PREP
DE101	AZUL Tissue DNA Extraction Kit	25/50 preps
DE102	AZUL Animal Cell Culture DNA Extraction Kit	25/50 preps
DE103	AZUL Bacterial DNA Extraction Kit	25/50 preps
DE104	AZUL Plasmid DNA Extraction Kit	25/50 preps
DE105	AZUL Plant DNA Extraction Kit	25/50 preps
DE106	AZUL Soil DNA Extraction Kit	25/50 preps
DE107	AZUL Blood DNA Extraction Kit	25/50 preps
DE108	AZUL Cell-free DNA Extraction Kit	25/50 preps
DE109	AZUL DNA Extraction Kit- Difficult samples	25/50 preps
DE110	AZUL Saliva DNA Extraction Kit	25/50 preps
DE111	AZUL Stool DNA Extraction Kit	25/50 preps
DE112	Quick AZUL Bacterial/Fungal DNA Extraction Kit	25/50 preps
DE113	AZUL Microbiome DNA Extraction Kit	25/50 preps
DE114	AZUL Gel DNA Extraction Kit	25/50 preps
DE115	AZUL FFPE DNA Extraction Kit	25/50 preps
DE116	AZUL Chloroplast DNA Extraction Kit	25/50 preps
DE117	AZUL Mitochondrial DNA Extraction Kit	25/50 preps
DE118	AZUL Pollen DNA Extraction Kit	25/50 preps
DE119	AZUL Fungal DNA Extraction Kit	25/50 preps
DE120	AZUL Sperm DNA Extraction Kit	25/50 preps
DE121	AZUL Skin DNA Extraction Kit	25/50 preps

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FEEDBACK

How did this kit perform?

Did AZUL Extraction Kit fulfill expectations required for your research?
Let us know by filling out the feedback form here

Or scan the QR code:

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