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A Geno Technology, Inc. (USA) brand name

XIT™ Mitochondrial DNA

For the Isolation of Mitochondrial DNA from Mammalian Cells & Tissue

INTRODUCTION

The *XIT*™ Mitochondrial DNA kit is designed for the isolation of mitochondrial DNA from mammalian cells and tissue. The *XIT*™ kit uses mitochondrial isolation followed by lysis, protein digestion and precipitation and finally DNA precipitation to isolate high quality mitochondrial DNA.

XIT™ Mitochondrial DNA kits are offered for the processing of a maximum of $20x10^8$ cells. Alternative protocols are supplied for processing mitochondrial DNA from soft tissue (brain or liver) and hard tissue (skeletal or heart muscle).

ITEMS SUPPLIED Cat. # 786-301

Description	Size
SubCell Buffer-I	60ml
SubCell Buffer-II [3X]	30ml
Mitochondrial Lysis Buffer	60ml
LongLife™ Proteinase K (5mg/ml)	0.5ml
XIT™ Protein Precipitation Buffer	25ml
TE Buffer	10ml
LongLife™ RNase	0.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. After receiving store the kit components at 4°C. A precipitate may form in the buffers, which is readily removed by gently warming to room temperature. The kit is stable for one year when stored unopened. Use aseptic techniques when handling the reagent solutions.

ITEMS NEEDED BUT NOT SUPPLIED

- Syringes and 20 gauge needles or Wheaton Dounce homogenizer
- Centrifuge and centrifuge tubes
- Optional reagents: Delipidated BSA, Trypsin, PBS and Protease Inhibitor Cocktail (e.g. Protease Arrest, Cat# 786-108)

PREPARATION BEFORE USE

Chill both SubCell Buffers on ice before using. Dilute appropriate volume of 3X SubCell Buffer-II to 1X with SubCell Buffer-I as needed (e.g. mix 2ml SubCell Buffer-I with 1ml SubCell Buffer-II). All centrifugation steps should be performed at 4°C. Warm Mitochondrial Lysis Buffer to room temperature before use.

PROTOCOL: Isolation of Mitochondria from Animal Cells.

This protocol is for processing $20x10^6$ cells (or ~100µl wet cell pellet). It can be scaled up and down accordingly.

1. Use fresh cells only. Pellet the harvested cells by centrifugation at ~800 x *g* for 1 minute. Carefully remove and discard the supernatant.
   OPTIONAL: *Wash the cell pellet with 1ml ice cold PBS, centrifuge it as above and discard the supernatant.*
2. Add 500µl of ice cold SubCell Buffer-I. Gently vortex to suspend the cells and incubate on ice for 10 minutes.

3. Perform this lysis step on ice. Using a narrow opening (20 gauge) syringe needle, gently pull the suspension up and down 10-30 times.

   NOTE: *Alternatively, suspend cells in 300µl ice cold SubCell Buffer-I, transfer cell suspension to ice cold Dounce homogenizer. Homogenize the cells on ice using tight pestle. Perform 5 to 20 strokes to lyse the cells effectively. Transfer the lysate to a microcentrifuge tube. Rinse Dounce homogenizer with 200µl of SubCell Buffer-I and pool together. Invert the tube several times to mix.*

   NOTE: *To check the cell lysis efficiency, spot 5µl of cell lysate onto a glass slide, add coverslip and view under a phase-contrast microscope. Pulling times or strokes in the above lysis step are only guidelines. Mechanical force to lyse cells depends on cell types, the total number of the cells and hands on experience. Insufficient force will not lyse all the cells, but will achieve cleaner mitochondrial fractions with less nuclear contamination. Excess force may damage some nuclei, but high yield mitochondria fractions will be obtained with some contamination from nuclei.*
4. Add 250µl 3X SubCell Buffer-II (350µl if Dounce homogenizer is used) and mix by inverting. This generates a 1X final concentration of SubCell Buffer-II.
5. Centrifuge the tube at 700x *g* for 10 minutes to pellet the nuclei. Transfer the supernatant to a new tube.
6. Centrifuge supernatant at 12,000x *g* for 15 minutes. The pellet contains mitochondria.
7. Lyze the mitochondrial pellet in 500µl Mitochondrial Lysis Buffer and incubate at room temperature for 10-15 minutes.
8. Add 5µl *LongLife*™ Proteinase K to the tube and mix by inverting the tube 10-20 times. Incubate at 55°C for at least 2 hours. The incubation can be extended overnight for maximal yield. Invert the tube periodically during the incubation.
9. After incubation, incubate the sample on ice for 1 minute to quickly cool. Do not store on ice.
10. Add 100µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
11. Centrifuge at 14,000g for 2 minutes. Carefully, transfer the supernatant to a fresh tube.

    NOTE: *The precipitated protein should form a tight white pellet. If not, incubate the sample on ice for 5 minutes and repeat the centrifugation.*
12. Add 500µl isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.

    NOTE: *If DNA concentrations is expected to be low (<1µg), add 1µl Mussel Glycogen Solution.*
13. Centrifuge at 14,000g for 5 minutes.
14. Discard the supernatant and use a pipette to carefully remove excess liquid.
15. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
16. Centrifuge at 14,000g for 2 minutes.
17. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
18. Add 50µl prewarmed TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
19. Rehydrate the genomic DNA by incubating at 55-65°C for one hour, followed by an overnight incubation at room temperature to ensure complete genomic DNA hydration.
20. Store DNA at 4°C, for long term storage store at -20 or -80°C.

APPENDIX

A. Isolation of mitochondria from soft tissues (liver or brain)

OPTIONAL: *Delipidated BSA can be added to 1X SubCell Buffer-II to the concentration of 2mg/ml for removing free fatty acids prior processing.*

1. Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. Do not freeze.
2. Weigh approximately 50-100mg tissue. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.
3. Perform this step on ice. Transfer the minced tissue to an ice-cold Dounce tissue homogenizer. Add 10 volumes of 1X SubCell Buffer-II and using a loose-fitting pestle disaggregate the tissue with 5-10 strokes or until the tissue sample is completely homogenized. Using a tight-fitting pestle, release the nuclei with 8-10 strokes. Do not twist the pestle as nuclei shearing may occur.

4. Stand on ice for 2 minutes. Transfer the homogenate to a centrifuge tube and leave large chunks of tissue fragments in the homogenizer to be discarded. Centrifuge the lysate at 700x *g* for 5 minutes to pellet the nuclei.
5. Carefully transfer the supernatant into a new tube. Centrifuge supernatant at 12,000x *g* for 10 minutes.
6. Remove the supernatant and resuspend the pellet in 10 volumes of 1X SubCell Buffer-II without BSA.
7. Centrifuge as in step 4. Repeat step 5 and remove the supernatant. The pellet contains mitochondria.

B. Isolation of mitochondria from hard tissues (skeletal or heart muscle)

NOTE: *For facilitating homogenization of the hard tissue, 0.25mg/ml Trypsin should be added to 1X SubCell Buffer-II. A concentrated BSA solution is needed to quench the proteolytic reaction after Trypsin treatment.*

1. Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. Do not freeze.
2. Weigh approximately 50-100mg tissues. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.
3. Suspend the sample with 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml trypsin in a 2ml centrifuge tube.
4. Incubate on ice for 3 minutes and then spin down the tissue for a few seconds in the centrifuge.
5. Remove the supernatant by aspiration and add 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml Trypsin. Incubate on ice for 20 minutes.
6. Add BSA Solution to a final concentration of 10mg/ml and mix. Spin down the tissue at 1,000 x g for 5-10 seconds in the centrifuge.
7. Remove the supernatant by aspiration. Wash the pellet with 8 volumes of 1X SubCell Buffer-II without Trypsin, and spin down the tissue for a few seconds in the centrifuge.
8. Remove the supernatant by aspiration and add 8 volumes of the 1X SubCell Buffer-II without Trypsin.
9. Transfer the suspension to an ice-cold Dounce tissue homogenizer and using a loose-fitting pestle disaggregate the tissue with 5-15 strokes or until the tissue sample is completely homogenized. Using a tight-fitting pestle, release the nuclei with 8-10 strokes. Do not twist the pestle as nuclei shearing may occur.
10. Stand on ice for 2 minutes. Transfer the homogenate to a centrifuge tube and leave large chunks of tissue in the homogenizer to be discarded. Centrifuge the lysate at 700 x *g* for 5 minutes to pellet nuclei.
11. Transfer the supernatant to a new tube. Centrifuge it at 12,000x*g* for 10 minutes and remove the supernatant. The pellet contains mitochondria.

RELATED PRODUCTS

1. ***EZ-Grind™ (Cat # 786-139)****: A highly efficient grinding resin that is pre-aliquoted into 1.5ml grinding tubes and is supplied with matching pestles.*
2. ***Pestle & Tubes (Cat. # 786-138P):*** *DNase/RNase free microfuge tubes (1.5ml) and matching pestles for the grinding of small samples and isolation of nuclei.*
3. ***Molecular Grinding Resin™ (Cat # 786-138):*** *For grinding of small samples. High tensile micro particles that do not bind nucleic acids.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*

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