Jun 23, 2020

Mitochondrial Isolation SOP (#10)

DOI
dx.doi.org/10.17504/protocols.io.bfr7jm9n

Elena Pumbo[1], Kimberly Chapman[1]

[1]Children's National Rare Disease Institute

Kimberly Chapman
Children's National Rare Disease Institute

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bfr7jm9n

Protocol Citation: Elena Pumbo, Kimberly Chapman 2020. Mitochondrial Isolation SOP (#10). **protocols.io https://dx.doi.org/10.17504/protocols.io.bfr7jm9n**

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

Created: April 29, 2020

Last Modified: June 23, 2020

Protocol Integer ID: 36383

Keywords: mitochondrial isolation,

Abstract

Protocol for mitochondrial isolation used by our group for assays needing intact and mitochondrial lysates.

Attachments

SOP_#010_Mitochondri..

156KB

Tissue Prepration

1 **Tissue preparation**

2 For 5 mL of 15-20 mg/mL of liver mitochondria protein we need 10-15 gm of frozen mouse liver tissue. If more of less protein needed scale these numbers up or down.

3 Cool down centrifuge and Rotor F-35-6-30 for 6 × 50 mL and 5 × 15 mL tubes. Set temperature +4^{0}C.

4 ·Take livers from -80^{0}C freezer put on dry ice.

5 ·Measure weight of a 10×10 cm square of aluminum foil using Mettler Toledo Analytical Balance and write it down.

6 ·Put on the foil unwrapped frozen livers up to 15 gm (about 10 livers)

7 ·Subtract foil weight.

8 ·Write number.

9 ·Keep livers on ice.

10 ·Bring liquid nitrogen in special 1 L liquid nitrogen container.

11 ·Take ceramic mortar and pestle

12 ·Fill mortar with liquid nitrogen and put pestle on dry ice cool down.

13 ·Put metal spatula on dry ice to cool down.

14 ·After liquid nitrogen evaporate mortar is cold enough.

15 ·Prepare 50 mL conical tube and put int on dry ice

16 ·Put 1st frozen liver in the mortar and add ½ liquid nitrogen up to ½ mortar volume.

17 ·Wrap pestle with paper towel to avoid frost bite and cover mortar with paper towel.

18 ·Grind liver till there is only powder in the mortar.

19 ·With cold spatula put grinded tissue in the 50 mL tube.

20 ·Repeat with next liver until all of them are ground.

21 ·Put mortar and pestle aside to warm up.

22 ·Clean the bench with 70% ethanol.

23 ·Prepare fresh 50 mL conical tube.

24 ·Measure weight using Mettler Toledo Analytical Balance and wright it down.

25 ·Transfer in fresh tube 10 gm of grinded liver powder (subtract weight of empty tube from weight of the tube with tissue).

26 ·Extra grinded liver store in -80^{0} C.

27 ·To the tube with 10 gm of frozen liver add Tissue lysis Buffer up to 50 mL.

28 ·Wait till all tissue thawed. Vortex from time to time.

29 ·Prepare 25 Matrix A 2mL tubes for MP Homogenizer

30 ·When tissue is fully thawed using transfer pipettes aliquot 50 mL in Martix A tubes.

31 ·Following instruction for MP Homogenizer choose on touch screen

32 -Recommended Settings (Animal: mouse, organ: liver)

33 -Run

34 ·After program finished put tubes with homogenized liver on regular ice.

35 ·Clean and turn off MP Homogenizer

36 ·Using transfer pipette transfer homogenate into fresh 50ml tube.

37 **2. Mitochondria Extraction**

38 ·Prepare 50 mL conical tube with water for balance

39 ·Using transfer pipette transfer homogenate from Matrix A tubes into fresh 50ml tube.

40 ·Label tube “Pellet 1”

41 ·Put 50 mL tube with homogenate and balance into Rotor F-35-6-30

42 **1st slow centrifugation. Close rotor lid tightly**
Close centrifuge
Set centrifuge: 2000 rpm, 10 min, 4 °C

43 ·After centrifugation finished, take tube from centrifuge and put on ice.

44 ·Transfer supernatant into fresh tube. Label tube “Supernatant 1”

45 ·Discard pellet.

46 ·Adjust balance volume to new sample volume.

47 ·Put 50 mL tube with homogente and balance into Rotor F-35-6-30

48 **1st fast centrifugation. Close rotor lid, tightly**
Close centrifuge, Set centrifuge: 7830 rpm, 20 min, 4 °C

49 ·After centrifugation finished, take tube from centrifuge and put on ice.

50 ·With q-tips remove white fat floating on the top of supernatant.

51 ·Discard supernatant.

52 ·Resuspend pellet in 25 mL of Tissue Lysis Buffer.

53 ·Transfer into fresh tube. Label tube “Pellet 2”

54 ·Adjust balance volume to new sample volume.

55 ·Put 50 mL tube with homogenate and balance in Rotor F-35-6-30.

56 **2st slow centrifugation: Close rotor lid tightly, Close centrifuge.**
Set centrifuge: 2000 rpm, 10 min, 4 °C

57 ·After centrifugation finished, take tube from centrifuge and put on ice.

58 ·Transfer supernatant into fresh tube. Label tube “Supernatant 2”

59 ·Discard pellet.

60 ·Adjust balance volume to new sample volume.

61 **2st fast centrifugation: Close rotor lid tightly, close centrifuge.**
Set centrifuge: 7830 rpm, 20 min, 4 °C

62 ·After centrifugation finished, take tube from centrifuge and put on ice.

63 ·Change rotor for Rotor FA-45-48-11 for 48 2 mL tubes.

64 ·With q-tips carefully remove fat floating on top of supernatant.

65 ·Discard supernatant.

66 ·Resuspend pellet in 12 mL of Tissue Lysis Buffer.

67 ·Transfer for 2 mL into fresh 2 mL microcentrifuge tubes.

68 ·Load rotor distributing tubes equally.

69 **Wash centrifugation: Close rotor lid tightly, close centrifuge,**
Set centrifugre: 12700 rpm, 20 min, 4 °C

70 ·After centrifugation finished, take tube from centrifuge and put on ice.

71 ·Discard supernatant.

72 ·Pellets resuspend in 5-6 mL of Tissue Lysis Buffer and transfer into 15 mL conical tube.

73 ·Put back Rotor F-35-6-30 for 15 and 50 mL tubes.

74 ·Set centrifuge: 7830 rpm, 20 min, 4 °C

75 ·After centrifugation finished, take tube from centrifuge and put on ice.

76 ·Discard supernatant.

77 **3. Mitochondria Protein Extraction**

78 Prepare 10 mL of Cell Lysis Buffer with 1 tablet of Proteinase Inhibitor (PI) cocktail.

79 ·Label tube with preparation date.

80 Using 5mL serological pipette add to mitochondria pellet up to 5 mL of Cell Lysis Buffer.

81 ·Using

82 Using 200 µL pipettor with 200 µL pipette tip add 50 µL of Triton X-100.

83 ·Pipette up and down 20 times to make sure all Triton X-100 was washed from the tip.

84 ·Resuspend the pellet.

85 Incubate 15 min 4 °C (in cold room) with rotation.

86 ·Transfer 2 mL into fresh 2 mL microcentrifuge tubes.

87 ·Change rotor for Rotor FA-45-48-11 for 48 × 2 mL tubes.

88 ·Load rotor distributing tubes equally.

89 ·Close rotor lid tight.

90 ·Close centrifuge.

91 ·Set centrifuge: 12,700 rpm, 20 min 4 °C

92 ·After centrifugation finished, take tube from centrifuge and put on ice.

93 ·Transfer supernatant into 5 mL tube.

94 Next step – protein concentration measurement. Summar Lab SOP #4 “Protein Quantification by Bradford Assay”