Nuclei Extraction for tissue using Iodixanol Gradients V.1

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Version 1

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ABSTRACT

Nuclei isolation using Iodixanol gradients geared for multiome

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Creating Buffers

1 Stock Buffers

All stock solutions should be filtered using a .22 um PVDF/PES filter system. All solutions except 50% Iodixanol solution are stable at 4c for at least 6 months.

1 M Sucrose (300 mL)

Substance:	Stock Conc.:	Amount:	Final conc. in working solution:
Sucrose	-	102.69 g	1 M
Water	-	235.5 mL	-

1.0616x Homogenization Buffer Stable Solution (200 mL)

Substance:	Stock conc:	Amount:	Final conc. in working solution:
Sucrose	1 M	53.1 mL	.2653 M
KCl	2 M	2.66 mL	26.6 mM
MgCl2	1 M	1.06 mL	5.31 mM
Tricine-KOH pH 7.8	.75 M	5.67 mL	21.2 mM
Water	-	137.5 mL	-

Diluent Buffer (100 mL)

Substance:	Stock conc:	Amount:	Final conc. in working solution:
KCl	2 M	7.5 mL	150 mM
MgCl2	1 M	3 mL	30 mM
Tricine KOH pH 7.8	.75 M	16 mL	120 mM
Water	-	73.5 mL	-

50% Iodixanol Solution (50 mL) **Remake Monthly for Stability

Substance:	Stock conc:	Amount:	Final conc. in working solution:
Diluent Buffer	-	8.3 mL	-
Iodixanol	60%	41.7 mL	50%

1x Homogenization Buffer Unstable Solution for 4 reactions **Prepare Fresh**

Substance:	Stock conc:	Amount:	Final conc. in working solution:
HB stable solution	1.0616X	7536 uL	1X
DTT	1 M	8 uL	1 mM
Spermidine	500 mM	8 uL	.5 mM
Spermine	150 mM	8 uL	.15 mM
NP40	10%	240 uL	.3%
cOmplete PI (diluted in HB stable)	100X	80 uL	1X
Ribolock	40U/uL	120 uL	.6 U/uL

30% Iodixanol Solution per reaction **Prepare Fresh**

Substance:	Stock conc:	Amount:	Final conc. in working solution:
HB unstable	-	240 uL	-
50% Iodixanol Solution	50%	360 uL	30%

40% Iodixanol Solution per reaction **Prepare Fresh**

Substance:	Stock conc:	Amount:	Final conc. in working solution:
HB unstable	-	120 uL	-
50% Iodixanol Solution	50%	480 uL	40%

Wash Buffer 1 mL for 4 reactions **Prepare Fresh**

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Substance:	Stock conc:	Amount:	Final conc. in working solution:
Tris-HCl pH 7.4	1 M	10 uL	10 mM
NaCl	5 M	2 uL	10 mM
MgCl2	1 M	3 uL	3 mM
BSA	30%	33.3 uL	1%
Tween-20	10%	10 uL	.1%
DTT	1 M	1 uL	1 mM
Ribolock	40 U/uL	15 uL	.6 U/uL

2 Before starting protocol:

1) pre-chill swinging bucket centrifuge and a fixed angle centrifuge to 4c
2) Pre-chill dounces and pestles to 4c on ice
3) Pre-chill tubes
4) Fill up 2 L beaker with 500 mL sterile water to soak the used Dounces

3 Isolation of Nuclei via Dounce Homogenization and Density Centrifugation

1) place 20-50 mg frozen tissue or crushed into pre-chilled 7 mL dounce containing 1 mL cold 1x HB
2) Dounce with "A" loose pestle until resistance goes away (~10 strokes)
3) Dounce with "B" tight pestle until resistance goes away (~15 strokes)
4) Place "A" and "B" into sterile water to soak for cleaning later
5) Filter during transfer into FACS tube
6) Place Dounce into beaker with sterile water to soak for cleaning later
7) Pellet nuclei by spinning 5 min at 4c at 350 xg in a fixed angle centrifuge
8) Remove 950 uL of supernatant (50 uL remaining)
9) Gently resuspend nuclei in 350 uL 1x HB
10) Add 1 volume (400 uL) of 50% Iodixanol solution and pipette mix
11) Slowly layer 600 uL of 30% Iodixanol solution under the 25% mixture. Wipe side of pipette tip with kimwipe to avoid mixing layers.
12) Layer 600 uL 40% Iodixanol solution under the 30% mixture. Wipe side of pipette tip with kimwipe to avoid mixing layers.
13) In a pre-chilled swinging bucket centrifuge, spin for 20 min at 4c at 3000 xg with the brake off. Set acceleration level to 1 and deceleration at 0. (centrifuge time=23 min, time to stop=13 min)
14) Slowly extract top layers in increments of 200 uL down to 200-300 uL of nuclei band between 30% and 40% interface.
15) Take 200 uL of the nuclei band and put into 1.5 mL LoBind tube
16) Dilute nuclei by adding 200 uL of wash buffer and mix by pipetting
17) Count nuclei using trypan blue
18) Centrifuge at 500 xg for 5 min at 4c

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19) Remove supernatant without disrupting pellet
20) resuspend in x uL of chilled Nuclei Buffer (depending on what isolated nuclei are used for) to achieve target concentration based on count.

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