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Nuclei Isolation from Tissue for 10x snRNA

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1 Works for me This protocol is published without a DOI.

HubMAP

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ABSTRACT

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MATERIALS TEXT

Stock Buffers

All stock solutions should be filtered using a 0.22 um PVDF filter system. All solutions except for the 50% Iodixanol solution are stable at 4°C for at least 6 months.

1.034x Homogenization Buffer Stable Solution		For 200 ml stock solution		
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Total Vol. (ul)*
1	M Sucrose	0.26	3.87	51706.50
2	M KCl	0.03	77.36	2585.33
1	M MgCl2	0.01	193.40	1034.13
0.75	M Tricine-KOH pH 7.8	0.02	36.26	5515.36
-	Water	-	-	139158.69
			Total Vol. (ul)	200000.00

Diluent Buffer		For 100 ml stock solution		
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Total Vol. (ul)*
2	M KCl	0.15	13.33	7500.00
1	M MgCl2	0.03	33.33	3000.00
0.75	M Tricine-KOH, pH 7.8	0.12	6.25	16000.00
-	Water	-	-	73500.00
			Total Vol. (ul)	100000.00

50% Iodixanol Solution		For 50 ml stock solution		
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Total Vol. (ul)*
-	Diluent Buffer	1	-	8333.33
60	% Iodixanol	50	1.20	41666.67
**Remake monthly for stability			Total Vol. (ul)	50000.00

ATAC-RSB Buffer		For 500 ml stock solution		
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Total Vol. (ul)*
1	M Tris-HCl pH 7.5	0.01	100.00	5000.00
5	M NaCl	0.01	500.00	1000.00
1	M MgCl2	0.003	333.33	1500.00
-	Water	-	-	492500.00
			Total Vol. (ul)	500000.00

1M Sucrose		For 300 ml stock solution		
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Total*
-	Sucrose (Powder)	1000	-	102.69 g
	H2O			235.5 ml
			Total Vol. (ul)	300000.00

Same Day Buffers – should be prepared fresh each day

**Note – cOmplete Protease Inhibitors come as tablets. It is difficult to use less than 1/2 tablet so we prepare the 1x Homogenization Buffer Unstable Solution in batches of 12 as outlined below.

1x Homogenization Buffer Unstable Solution				
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Vol per 12 samp. (ul)*
1.0341	x HB Stable Solution	1	1.03	24175.00
1	M DTT	0.001	1000.00	25.00
500	mM Spermidine	0.5	1000.00	25.00
150	mMSpermine	0.15	1000.00	25.00
10	% NP40	0.3	33.33	750.00
-	cOmplete Protease Inhibitor	-	-	0.50 Tablets
			Total Volume (ul)	25000.00

30% Iodixanol Solution				
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Vol per sample (ul)*
-	1x Homog. Buffer Unstable	-	-	240.00
50	% Iodixanol Solution	30	1.67	360.00
			Total Volume (ul)	600.00

40% Iodixanol Solution				
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Vol per sample (ul)*
-	1x Homog. Buffer Unstable	-	-	120.00
50	% Iodixanol Solution	40	1.25	480.00
			Total Volume (ul)	600.00

ATAC-RSB-Tween Buffer				
Stock	*Name*	*Final Conc.*	*Fold Dilution (x)*	*Vol per sample (ul)*
-	ATAC-RSB	-	-	2970.00
10	% Tween-20	0.1	100.00	30.00
			Total Volume (ul)	3000.00

ATAC-seqRxn Mix	
Reagent	*Vol per sample (ul)*
H2O	5
PBS	16.5
2x TD	25
1% Digitonin	0.5
10% Tween-20	0.5
Tn5	2.5

Order List

Item	Supplier	Cat Number
Eppendorf 2 ml Lo-Bind tubes	Sigma	Z666556-250EA
Eppendorf 1.5 ml Lo-Bind tubes	Sigma	Z666548-250EA
Nunc cryovials	Thermo	375418PK
Iodixanol (comes at 60%)	Sigma	D1556-250ML
Sucrose	Sigma	S7903-250G
NP40	Roche (Sigma)	11332473001
Tricine	Sigma	T0377-25G
Potassium Hydroxide (KOH)	Sigma	P5958-250G
cOmplete Protease Inhibitors	Roche	11697498001
MgCl2	Ambion (Thermo)	AM9530G
KCl	Ambion (Thermo)	AM9640G
DTT	Thermo	R0861
Spermidine	Sigma	S2501
Spermine	Sigma	S3256-1G
70 um Flowmi cell strainers	Fisher	03-421-228
70 um bucket-style cell strainers	BD Falcon	352350
Tris-HCl pH 7.5	Invitrogen	15567-027
NaCl	Ambion (Thermo)	AM9759
Tween 20	Roche (Sigma)	11332465001
H2O	Invitrogen	10977-015
Dounce Tissue Grinder Set	Sigma	D8938-1SET
INCYTO Disposable hemocytometers	Fisher	22-600-100
BAM Banker	Wako Chemicals	302-14681
RiboLock	Thermo	EO0384
0.22 um PVDF Filter Units (500 ml)	Millipore	SCGVU05RE
0.22 um PVDF Filter Units (50 ml)	Millipore	SE1M179M6

Before you start the protocol:

1 1)All steps should be performed on ice or at 4°C. Pre-chill a swinging bucket centrifuge and a fixed angle centrifuge to 4°C.
2)Pre-chill all Dounces and pestles to 4°C in a fridge.
3)Pre-chill all tubes. For each sample you are processing, you will need:
a.One 2 ml round-bottom LoBind tube for gradient separation
b.One 50 ml conical for filtration step (often optional)
4)Prepare all buffers. For faster dissolution, crush protease inhibitor tablets prior to addition to 1x Homogenization Buffer Unstable Solution. DTT, Spermidine, Spermine, and digitonin are stored at -20°C. All other detergents and buffers are stored at 4°C.
a.Remember that the catalog number provided for iodixanol from Sigma comes as a 60% solution (not 100%).
5)Fill up a 2 L beaker with 500 ml sterile water to soak the used Dounces and pestles.

Isolation of Nuclei via Dounce Homogenization and Density Gradient Centrifugation:

2 1)Remove samples from liquid nitrogen storage and keep on dry ice until use.
2)If you are working with a tissue type that is particularly hard to dissociate via Douncing, it can be helpful to pre-crush (do not pulverize) your tissue fragment using mortar and pestle. The tissue must remain frozen and cold during this entire process. Otherwise proceed to Step 3.
3)Place 20-50 mg frozen tissue or crushed tissue into a pre-chilled 2 ml Dounce containing 1 ml cold 1x HB (add 15 ul RiboLock per ml of1x HB and mix well).
4)Dounce with “A” loose pestle until resistance goes away (~10 strokes).
5)Place “A” pestle into beaker with sterile water to soak for cleaning later.
a.Optional – If residual un-homogenized tissue makes it difficult to Dounce, filter homogenate through a pre-chilled 50 ml conical using a 70 um bucket-style cell strainer filter prior to using tight pestle “B”.
6)Dounce with “B” tight pestle until resistance goes away (~20 strokes).
7)Place “B” pestle into beaker with sterile water to soak for cleaning later.
8)Filter during transfer using a 40 um cell strainer (Fisher Scientific Cat: 22363547) and transfer homogenate to a pre-chilled 50 ml tube. Transfer homogenate to pre-chilled 2 ml LoBind tube.
9)Place Dounce into beaker with sterile water to soak for cleaning later.
10)Pellet nuclei by spinning 5 min at 4°C at 350 RCF in a fixed angle centrifuge.
11)Remove all supernatant, if the pellet is not clearly visible, you can leave 50 ul supernatant in the tube.
12)Gently resuspend nuclei in 350 ul 1x HB, but make sure the total volume of nuclei suspension is 400 ul. Make sure nuclei are fully resuspended without clumps.
13)Add 1 volume (400 ul) of 50% Iodixanol Solution and mix well by pipetting
14)Slowly layer 600 ul of 30% Iodixanol solution under the 25% mixture. To avoid mixing of layers, wipe the side of the pipette tip with a Kimwipe to remove excess Iodixanol solution from the external surfaces of the pipette tip.
15)Layer 600 ul of 40% Iodixanol solution under the 30% mixture. To avoid mixing of layers, wipe the side of the pipette tip with a Kimwipe to remove excessIodixanol solution from the external surfaces of the pipette tip.
a.During this step, you will need to gradually draw your pipette tip up to avoid overflowing the tube. However, the tip of your pipette must stay below the 30%-40% interface at all times.
16)In a pre-chilled swinging bucket centrifuge, spin for 20 min at 4°C at 3,000 RCF with the brake off. Handle tubes gently so as to not disturb the gradient.
a.Iodixanol is meant to be used at higher speeds (10,000 RCF) but high-speed swinging bucket centrifuges are not always readily available so we perform this step at 3,000 g and have not had any issues.
17)Using a vacuum, aspirate the top layers down to within 200-300 ul of the nuclei band at the 30%-40% interface. Be careful not to get too close as you will disrupt the nuclei band.
18)Using a 200 ul volume, collect the nuclei band and transfer to a fresh tube. Do not aspirate more than 200 ul at this step as this can cause you to take too much of the 40% layer which sometimes contains debris.
19)Dilute nuclei by adding 200 ul of wash buffer (1X PBS with 1.0% BSA and 0.2U/µl RNase Inhibitor).Mix gently by pipetting. Filter nuclei suspension to 1.5 ml LoBind tube with 20uM Mini-Strainer (PluriSelect Cat: 431002040).
20)Centrifuge at 500 rcf for 5 min at 4°C.
21)Remove supernatant, resuspend nuclei in 100 ul of wash buffer.
22)Determine the nuclei concentration using a Countess Automated Cell Counter or manual counting using microscope.
23)Dilute nuclei to 800-1000 nuclei /ul using nuclease free water.
24)Proceed immediately to Chromium Next GEM Single Cell 3’ Reagent Kits User Guide (CG000204)

Stock Buffers

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