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Isolation of the Region Around Locus Coeruleus for Single Nucleus RNA Profiling

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Protocol status: Working

We use this protocol and it's working

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Disclaimer

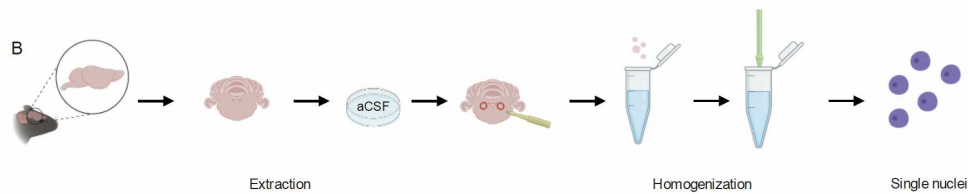
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Abstract

This protocol outlines the methodology for isolating the peri-locus coeruleus (peri-LC) region in mice for subsequent single nucleus RNA profiling. Key steps include the preparation of NMDG-aCSF buffer with precise pH and osmolarity, careful euthanasia and extraction of the brain, and the use of a vibratome to obtain 300 µm thick slices. The peri-LC region is identified and isolated using a biopsy puncher, followed by immediate shock-freezing in liquid nitrogen to preserve tissue integrity. This approach facilitates high-quality RNA profiling, essential for understanding the molecular characteristics of the locus coeruleus and its surrounding regions.

1 Tissue Isolation of the Region Around Locus Coeruleus for Single Nucleus RNA Profiling

2



After punching peri-LC region, you can also shock freeze in liquid nitrogen-

Buffer Preparation (important - steril filter and autoclave)

3 Prepare the buffer a day before, and keep it in the fridge before using

4



reagent	[c]	Mass [g]	V (taken from 1M Stock)
NMDG	96 mM	18,74 g	
HCl	96 mM		5 ml
NaHCO ₃	25 mM	2,10025	30 ml
Glucose	25 mM	4,50	
HEPES	20 mM	4,7662	20 ml
N-acetylcystein	12 mM	0,97914	
MgSO ₄ -heptahydrate	10 mM	2,4647	10 ml
Sodium L-ascorbat (Ascorbinsäure)	5 mM	0,99	
Myo-Inositol (Cyclohexanhexol)	3 mM	0,5406	
Sodium Pyruvat	3 mM	0,33 g – 30 ml	
KCl	2,5 mM	0,1864	2,5 ml
Thiourea	2,0 mM	0,15224	2 ml
NaH ₂ PO ₄ – monohydrate	1,25 mM	0,1724875	1,25 ml
CaCl ₂	0,5 mM	0,055495	500 µl
Taurine	0,01 mM	0,00125	
Actinomycin-d	1 µg/ml	1 mg	
TTX	0,1 µM		50 µl
DNQX	10 µM		1ml
APV	10 µM		1ml
Pronase (Streptomyces griseus)	1 mg/ml		



Preparation Stock Solution for NMDG- aCSF

	Stock	brauche für ACSF
1M HEPES	23,83 g in 100 ml	20 ml
1M MgSo ₄ 7H ₂ O	24,64 g in 100 ml	10 ml
1M NaH ₂ PO ₄	3,44 g in 25 ml	1,25 ml
1M Thiourea	1,903 g in 25 ml	2 ml
1M CaCl ₂ · 2 H ₂ O	1,837 g in 15 ml	500 µl
1M KCl	1,864 g in 25 ml	2,5 ml
1M NaHCO ₃	8,4005 g in 100 ml	30 ml

Stock solution to accurately prepare working solution!

- 5 ~ 500ml Milli-Q-Water
- 6 add reagents of Table 1
- 7 fill up until 1000 ml
- 8 sterile filter
- 9 Bubble with 95% O₂ and 5% CO₂ until pH is stable (approx. 15-20 min)
- 10 adjust pH with HCl → must have: 7,3-7,4 pH, 315 mOsm Can be stored at 4°C for a couple of days.
- 11 On the day of the experiment add freshly: + 10,0 g Trehalose+ 50 µl (0,1 µM) TTX+ 1 ml (200 µM) DNQX+ 1 ml (50 µM) APV+ 1 µg/ml actinomycin-d

Preparation for Brain Extraction

- 12 1. Prepare NMDG-aCSF
sterile filtered pH 7,3



- Important between 7,3 and 7,4!! 300 mOsm after Trehalose
+ 10 mg/ 1L Trehalose
- Stir while bubbling with 5 % CO₂ and 95 % O₂.
for 15- 20 Min and check pH 7,3

13 Clean EVERYTHING with RNase free!

14 Prepare FALCON 3.1 Falcon with ACSF and FBS (0,5%)

14.1 250 µl FBS + 50 ml aCSF → close with Parafilm to prevent gas leakage 3.2 Falcon with ACSF and Protease (1mg/ml)

14.2 30 mg Protease + 30 ml ACSF

15 Prepare chambers for recovery of brain slices

15.1 Chamber with NMDG-aCSF (RT): Recovery of Slices

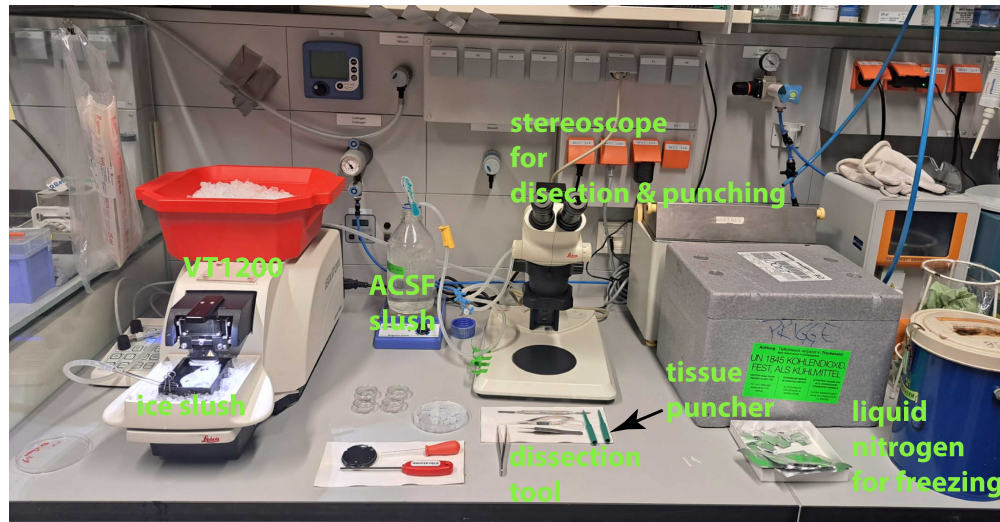
15.2 Chamber with RT NMDG-aCSF + Protease

16 Prepare Agaroseblock to assist vibrotome slicing and preventing pushing brain away during slicing
2% Agarose in Petri dish (fridge).
Cut out small block approx. 1,5 cm – 1,5 cm

17 Place Instruments on a towel for dissection and trituration → clean with RNase free

18 1. Prepare Vibratome: new blade each time!
80 Hz, 200 Frequency, Section: 300 µm (LEICA VT1200)

19



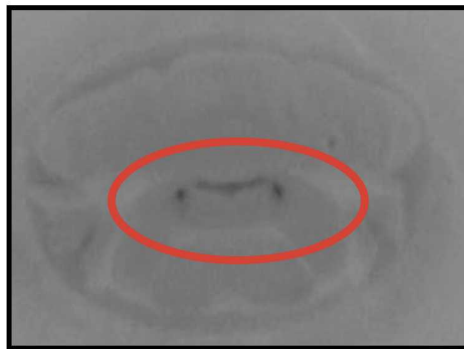
Procedure of extracting brain and cutting brain stem slices

- 20 Euthanize mice with 0.1 ml pento or other approved euthanizing method
- 21 Decapitate, remove skin, open skull (window)
- 22 Remove brain with spatula Block Brain (depending on region of interest (ROI))
- 23 Glue agarose block to platform Place and glue brain in front of agarose block
- 24 Place platform in vibratome, add ice cold NMDG-ACSF, bubble with 5 % CO₂ und 95 % O₂
- 25 Vibratome: 80 Hz, Amplitude 200, section 300 µm, duration 0,10 mm/s.
- 26 Discard brain slices until Locus coeruleus is reached

- 27 Place brain slices into ice-cold NMDG-ACSF bubble (5 % CO₂ und 95 % O₂) for 15 min
Recovery

Extracting and Isolating peri-LC region

- 28 1. Fill Petri dish with NMDG-aCSF+FBS
Place slices with pasteur pipette into petri dish
- 29 **When working with fluorescence labeled cells turn off light!**
- 30 Punch out brain areas with biopsy puncher (1.5 mm)
- 31 In this image the LC is accumulating NM and is easily visible. Landmarks such as the 4th ventricle can help you to locate LC and peri-LC region



Locus Coeruleus
initial section



Locus Coeruleus
mid section

- 32 After punching the peri-LC region, store the tissue in a small PCR tube and immediately shock-freeze it in liquid nitrogen. Ensure the tissue is sent to collaborators as intact tissue, **not** as isolated nuclei.



A biopsy tissue puncher with diameter of 1.5mm is recommended. We used biopuncher from Miltek (image copywrite Miltek/Tedpella

https://www.tedpella.com/histo_html/miltek-plunger-punch.aspx)

- 33 If you immediately proceed with nuclei isolation, follow the protocol provided by the 10X Nuclei Isolation Kit ([link](#))
- 34 Place samples on ice until further usage