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DEFND cryopreserved colon tissue

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Protocol status: Working
We tested this protocol, data analysis is still ongoing

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Abstract

This protocol details the description of DEFND cryopreserved colon tissue.

Materials

	A	B	C	D
	Colon lysis buffer	**Stock concentration**	**Final concentration**	**V (µL)**
	TrisHCl pH 7,4	1000 mM	10 %	10.1
	NaCl	5000 mM	10	2.0
	MgCl2	1000 mM	3	3.0
	DTT	100 mM	1	10.1
	RNase inhibitor RNasin (Promega)	40 U/µL	1	25.3
	Nuclease-free water			959.5
	Total volume			**1010.0**

	A	B	C	D
	Wash buffer Promega	**Stock concentration**	**Final concentration**	**V (µL)**
	TrisHCl pH 7,4	1000 mM	10 mM	22.2
	NaCl	5000 mM	10 mM	4.4
	MgCl2	1000 mM	3 mM	6.7
	BSA	10 %	1 %	222.0
	Tween-20	10 %	0.1 %	22.2
	DTT	100 mM	1 mM	22.2
	RNase inhibitor RNasin (Promega)	40 U/µL	1 U/µL	55.5
	Nuclease-free water			1864.8
	Total volume			**2220.0**

	A	B	C	D
	DEFND buffer	**Stock concentration**	**Final concentration**	**V (µL)**

	A	B	C	D
	TrisHCl pH 7,4	1000 mM	10 mM	2.2
	NaCl	5000 mM	10 mM	0.4
	MgCl2	1000 mM	3 mM	0.7
	Igepal (NP40 substitute)	10 %	0.1 %	2.2
	cOmplete protease inhibitor	25 X	1 X	8.8
	Pefabloc	20 mg/mL	1 mg/mL	11.0
	RNase inhibitor Protector (Sigma)	40 U/µl	0.5 U/µl	2.8
	Lithium diiodosalicylate (LIS)	100 mM	12.5 mM	27.5
	Nuclease-free water			164.5
	Total volume			**220.0**

	A	B	C	D
	Nuclei isolation buffer (NIB)	**Stock concentration**	**Final concentration**	**V (µL)**
	TrisHCl pH 7,4	1000 mM	10 mM	10.1
	NaCl	5000 mM	10 mM	2.0
	MgCl2	1000 mM	3 mM	3.0
	Igepal (NP40 substitute)	10 %	0.1 %	10.1
	cOmplete protease inhibitor	25 X	1 X	40.4
	RNasin (Promega)	20 U/µl	0.2 U/µl	10.1
	Nuclease-free water			934.3
	Total volume			**1010.0**

	A	B	C	D
	1X diluted nuclei buffer	**Stock concentration**	**Final concentration**	**Volume (µL)**
	20X nuclei buffer	20 X	1 X	5.0
	DTT	100 mM	1 mM	1.0

	A	B	C	D
	RNase inhibitor Protector (Sigma)	40 U/µL	1 U/µL	2.5
	Nuclease-free water			91.5
	Total volume			**100.0**

	A	B	C	D
	MNase reaction buffer	**Stock concentration**	**Final concentration**	**Volume (µL)**
	20X nuclei buffer	20 X	1 X	5.0
	CaCl2	1000 mM	5 mM	0.5
	DTT	100 mM	1 mM	1.0
	RNase inhibitor Protector (Sigma)	40 U/µL	1 U/µL	2.5
	Nuclease-free water			91.0
	Total volume			**100.0**

	A	B	C	D
	MNase 0,2U/µL	**Units in powder**	**Stock concentration**	**Volume (µL)**
	Lyophilised MNase in vial	50 U	0.2 U/µL	
	Nuclease-free water			250.0
	Total volume			**250.0**

	A	B	C	D
	Stop solution	**Stock concentration**	**Final concentration**	**V (µL)**
	EGTA	500 mM	100 mM	0.6
	Nuclease-free water			2.4
	Total volume			**3.0**

Remark: CaCl2 concentration may not be optimal, 5µL of 0,1M CaCl2 added to 500µL nuclei and 5µL enzyme is recomended. pH may also not be optimal, as it should be between 9 and 10.

Before start

Book necessary instruments and pre-chill centrifuge to 4°C.

Sectioning

1 Cool cryostat to -18 °C (blade and chamber).

2 Take sample out of -80 °C and place on dry ice.

3 Make 6-10 sections of 50 µm and place in DNA LoBind tube.

Homogenization

17m

4 1m

> **Note**
>
> Perform all following steps on wet ice.

Add 500 µL colon lysis buffer and incubate for 00:01:00 .

5 Pipet up and down 20 times.

6 Add an additional 500 µL colon lysis buffer.

7 Incubate On ice for 00:01:00 and pipet up and down 20 times. 1m

8 Triturate the sample 20 times using a pellet pestle.

9 Spin for 00:05:00 at 500 x g and 4 °C, remove the supernatant and resuspend in 1 mL wash buffer Promega. 5m

10 Transfer the sample with wide-bore pipette onto 40 µm strainer (pluriStrainer mini) placed on 2 mL DNA LoBind tube.

11 Wash the 1.5 mL tube with additional 500 µL wash buffer Promega, transfer onto the filter.

12 Spin the 2 mL tube for 00:05:00 at 500 x g and 4 °C . 5m

13 Remove the supernatant after the centrifugation.

14 Resuspend the pellet in 700 µL of wash buffer Promega.

15 Filtrate through flowmi and move to a 20 µm filter (pluriStrainer mini) placed on 2 mL DNA LoBind tube.

16 Centrifuge 00:05:00 at 500 x g and 4 °C . 5m

17 Remove the supernatant after the centrifugation.

Permeabilization 10m

18 Resuspend in 200 µL 12.5 mM DEFND buffer (start incubation time when resuspending).

19 Incubate On ice for 00:05:00 . 5m

20 Immediately following this incubation, add 1 mL of NIB and centrifuge the nuclei at 4 °C for 00:05:00 at 500 x g . 5m

21 Resuspend the pellet in either 25 µL 1X diluted nuclei buffer or 50 µL MNase reaction buffer (CaCl2), depending on choice in step 25 (full Multiome experiment or

nucleosome depletion MNase QC).

22 Dilute further if clumping is present and/or the concentration is too high.

23 Count nuclei using Propidium Iodide and Acridine Orange (AO/PI).

24 Nuclei count

Total cell concentration (cells and nuclei):
Live cell concentration (cells):
Dead cell concentration (nuclei):
Viability:

25 Either continue with the full 10X Genomics Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (CG000338 Rev F) protocol without alterations or perform a nucleosome depletion quality control using MNase (Merck), as instructed below.

> Note
>
> Adapted from Olsen TR, Talla P, Sagatelian RK, Furnari J, Bruce JN, Canoll P, Zha S, Sims PA. Scalable co-sequencing of RNA and DNA from individual nuclei. Nat Methods. 2025 Mar;22(3):477-487. doi: 10.1038/s41592-024-02579-x. Epub 2025 Feb 12. PMID: 39939719; PMCID: PMC12272635.

Digestion

2m

26 Transfer 50 µL (or other volume) of nuclei resuspend in reaction buffer to different eppendorf tubes, so split conditions from nucleosome depletion into two.

27 Preheat ThermoShaker to 28 °C .

28 Add desired volume of micrococcal nuclease solution (1 unit per 5 µL) to obtain 7 U of enzyme per 1×10^6 cells.

29 Mix gently and constantly for 00:02:00 in a 28 °C ThermoShaker.

2m

30 At the end of 2 minutes, add 1 µL (or other volume equal to 1/50 from sample volume) of [M] 0.1 Molarity (M) EGTA to stop.

31 Negative control reaction buffer and reaction buffer + stop solution without MNase.

DNA extraction

32 Add 100 µL RLT+ buffer to lyse nuclei and vortex.

33 Extract DNA using DNA Clean & Concentrator® -5 column following manufacturer's protocol.

Protocol references

Olsen TR, Talla P, Sagatelian RK, Furnari J, Bruce JN, Canoll P, Zha S, Sims PA. Scalable co-sequencing of RNA and DNA from individual nuclei. Nat Methods. 2025 Mar;22(3):477-487. doi: 10.1038/s41592-024-02579-x. Epub 2025 Feb 12. PMID: 39939719; PMCID: PMC12272635.