User Manual

Version 1.2 – UMNP4300

PARSE BIOSCIENCES
A QIAGEN company

Evercode™ FFPE Nuclei Preparation

For use with

ECNP4300

ECNP4500

Support Suite

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Overview

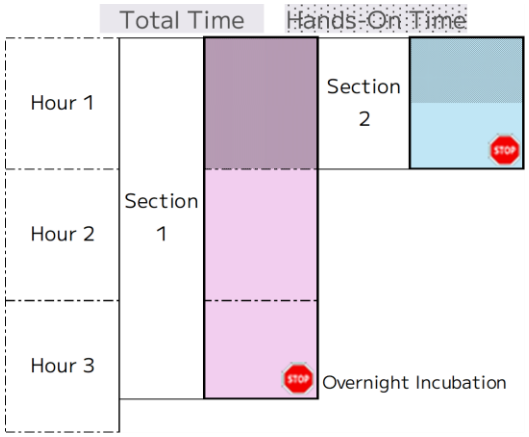
This FFPE nuclei preparation protocol is designed to isolate intact nuclei from formalin-fixed, paraffin-embedded (FFPE) tissues for single-nucleus RNA sequencing (snRNA-seq).

Protocol Timing

The table below provides details of the total and hands-on time required for the whole FFPE Nuclei Preparation workflow. We recommend starting this protocol in the afternoon to accommodate the overnight incubation step.

DESCRIPTION	TIME	HANDS-ON-TIME	STOPPING POINTS
Section 1: FFPE Nuclei Dissociation	170 min (plus 15-18 hrs incubation)	60 min	4°C ≤ 18 hours in the thermomixer.
Section 2: Nuclei Storage and Wash	60 min	30 min	-80°C ≤ 4 months.

Workflow



Important Guidelines

These guidelines provide additional information to obtain optimal performance beyond the detailed instructions in the protocol. For additional questions not discussed below, please contact us at support@parsebiosciences.com.

Sample Input

- This protocol begins with formaldehyde fixed & paraffin embedded (FFPE) blocks. We recommend cooling down tissue blocks on ice before sectioning.
- We recommend that a single operator process no more than 12 tubes per dissociation run. If additional samples need to be processed, perform the dissociation in separate batches.
- Nuclei yields from FFPE tissue blocks can vary based on tissue type, section surface area, and scroll thickness. Refer to the scroll input recommendation table in Section 1 to ensure sufficient nuclei yield per sample for each Evercode FFPE Nuclei Preparation Kit.

RNA Integrity

- We recommend performing an RNA quality assessment to determine the DV200 value, defined as the percentage of RNA fragments longer than 200 nucleotides, prior to running the Evercode WT FFPE assay. In general, higher DV200 values are associated with improved data quality. For optimal performance, we recommend using tissue blocks with a DV200 of at least 30%.

Reagent Stability

- Reagents in the FFPE Nuclei Preparation Reagents box should not be frozen and thawed more than 3 times.
- If using the kit more than 3 times, we recommend aliquoting the reagents into nuclease-free 1.5 mL tubes and storing them at -20°C until use. We do not recommend making single use aliquots to minimize the impact of evaporation during storage.
- Reagent master mixes should be made fresh and used the same day.

Avoiding RNase Contamination

- Standard precautions should be taken to avoid introducing RNases into samples or reagents throughout the workflow. Always wear proper laboratory gloves and use aseptic technique.

- Although RNases are not inactivated by ethanol or isopropanol, they are inactivated by products such as RNaseZap RNase Decontamination Solution (Thermo Fisher Scientific). These can be sprayed on benchtops and pipettes.
- Nuclease-free, filtered pipette tips should be used to reduce RNase contamination from pipettes.

Nuclei Counting and Quality Assessment

- For nuclei counting, a fluorescent counting device is needed.

- When first using an Evercode FFPE Nuclei Preparation workflow, we suggest saving images at each counting step. High quality dissociated nuclei have single distinct nuclei with <5% aggregation and no debris. Higher levels of aggregation will lead to elevated doublets after sequencing. When counting nuclei, it is critical to avoid counting cell debris to avoid overestimating the number of nuclei.

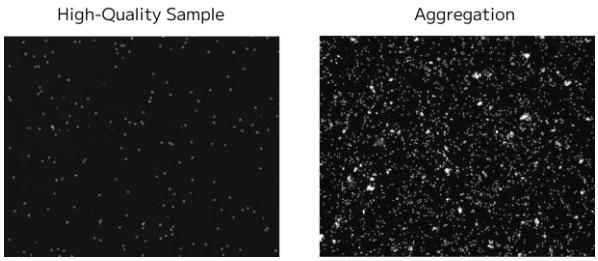


Figure 1: Example of AO fluorescent stained fixed nuclei.

Cell Strainers

- A cell strainer with an appropriately sized mesh should be used throughout the protocol. Although 20-30 µm is appropriate for many nuclei types, the mesh size should be chosen based on the sample type.

- To strain nuclei we recommend using the Miltenyi Pre Separation Filters as they use gravity for a gentle liquid run through.

Maximizing Nuclei Recovery

- It is critical to thoroughly resuspend the nuclei after centrifugation throughout the protocol. Resuspend by slowly and repeatedly pipetting until no clumps are visible. Ideally this should be verified with microscopy.

- We do not recommend wide bore pipette tips as they make it difficult to resuspend nuclei pellets adequately.

- Ensure that the 1.5 mL and 15 mL centrifuge tubes are polypropylene, as polystyrene tubes will lead to substantial sample loss.

Alternative Method: Using the Miltenyi Biotec FFPE Tissue Dissociation Kit for RNA Profiling

- While the manual dissociation procedure described in this user guide is the preferred method for FFPE nuclei preparation, the Evercode FFPE Nuclei Preparation kit is also compatible with automated dissociation approaches such as the Miltenyi Biotec FFPE Tissue Dissociation Kit for RNA Profiling. If using this kit, follow the manufacturer's protocol as written and stop at Section 2.2, Step 14, then continue with this protocol starting at Section 2, Step 15.

Parse Supplied Reagents

The Evercode FFPE Nuclei Preparation kit are available in two formats, 12-Reactions kit and 96-Reactions kit. Safety Data Sheets for these reagents can be provided upon request.

12-Reactions kit

-20°C Reagents Store -20°C, PN NP300

LABEL	ITEM	PN	FORMAT	QTY
Storage	Storage Buffer	NF102	2 mL tube	1
RNase Inhib	RNase Inhibitor	NF106	1.5 mL tube	1
DMSO	DMSO	NF107	1.5 mL tube	1
FFPE Resus Buff	FFPE Resuspension Buffer*	NF116	8 mL tube	1
FFPE Enhance	FFPE Enhancement Buffer*	NF117	1.5 mL tube	1

Note: *The FFPE Resuspension Buffer and FFPE Enhancement Buffer will not be used in this workflow. Save them for later use with the Evercode FFPE WT kit(s).

96-Reactions kit

-20°C Reagents Store -20°C, PN NP500

LABEL	ITEM	PN	FORMAT	QTY
Storage	Storage Buffer	NF302	8 mL tube	1
RNase Inhib	RNase Inhibitor	NF306	1.5 mL tube	1
DMSO	DMSO	NF307	1.5 mL tube	1
FFPE Resus Buff	FFPE Resuspension Buffer*	NF316	15 mL tube	1
FFPE Enhance	FFPE Enhancement Buffer*	NF317	15 mL tube	1

Note: *The FFPE Resuspension Buffer and FFPE Enhancement Buffer will not be used in this workflow. Save them for later use with the Evercode FFPE WT kit(s).

User Supplied Equipment and Reagents

The following materials and equipment are required to perform the protocol, but are not provided within the kit. Note that this list does not include standard laboratory equipment, such as freezers or fume hoods.

Equipment

ITEM	SUPPLIER	PN	NOTES
Centrifuge with Swinging Bucket Rotors	Various Suppliers	Varies	Compatible with 1.5 mL centrifuge tubes and capable of reaching 4°C and 10,000g.
Thermomixer	Various Suppliers	Varies	Capable of holding temperatures of 4°C and shaking up to 700 rpm, compatible with 1.5 mL tubes.
Fluorescence cell counter	Various Suppliers	Varies	Any fluorescence based cell counter such as EVE™ HT Automated Cell Counter.
PCR tube rack	Various Suppliers	Varies	Capable of holding semi-skirted 96 well PCR plates and a tight fitting lid.
Single Channel Pipettes: P20, P200, P1000. 12-channel: P20, P200	Various Suppliers	Varies	Or 8-channel pipettes can be substituted for 12-channel pipettes.
Styrofoam Cooler	Various Suppliers	Varies	

Consumables

ITEM	SUPPLIER	PN	NOTES
Miltenyi Pre-Separation Filters	Miltenyi Biotec	130-101-812 130-041-407	Choose one or an equivalent sterile cell strainer with an appropriate mesh size for the nuclei type(s) being fixed (20 µm, 30 µm). We do not recommend FlowMi Cell Strainers (SP Bel-Art).
DNA LoBind®Tubes, 1.5 mL, Snap Cap	Eppendorf®	022431021	Or equivalent DNA low-binding, nuclease-free 1.5 mL tubes.
Falcon® High Clarity PP Centrifuge Tubes, 15 mL	Corning®	352097	Or equivalent 15 mL polypropylene centrifuge tubes. Do not substitute polystyrene centrifuge tubes, as it will lead to substantial cell loss.

ITEM	SUPPLIER	PN	NOTES
Pipette Tips TR LTS 20 µL, 200 µL, 1000 µL	Rainin®	17014961 17014963 17014967	Or appropriate DNA low-binding, DNase/RNase-free, and filtered pipette tips. Do not use wide bore tips.
Pasteur pipettes	Various Suppliers	Varies	
Pasteur pipettes bulbs	Various Suppliers	Varies	
RNase-Free 1.5mL Disposable Pestle	Fisherbrand™	12-141-364	Or equivalent RNase-free pellet pestles.

Reagents

ITEM	SUPPLIER	PN	NOTES
RNaseZap™ RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	Or equivalent RNase decontamination solution.
Ethyl alcohol, Pure	Sigma-Aldrich	459844	Or equivalent 100% non-denatured ethanol.
Xylene	Sigma-Aldrich	534056	Or equivalent histological grade
N-Lauroylsarcosine sodium salt solution	Sigma-Aldrich	L7414	
Proteinase K	Thermo Scientific™	EO0491	
TRIS (1M), pH 8.0, RNase-free	Thermo Fisher Scientific	AM9855G	
EDTA 0.5M	Various Suppliers	Varies	
PBS	Various Suppliers	Varies	
Nuclease-Free Water	Sigma-Aldrich	W4502	Or equivalent nuclease-free water.

Section 1: FFPE Nuclei Dissociation

Prior to proceeding with the Evercode™ WT FFPE kits, nuclei are isolated from FFPE scrolls or blocks through xylene deparaffinization, ethanol rehydration, and tissue dissociation. This section concludes with a required overnight incubation at 4°C, which must be completed within 18 hours prior to starting the next section.

1. Set the thermomixer to 4°C.

2. Using the table below, determine the number of scrolls required for dissociation based on the kit type and the number of samples planned for downstream processing.

INPUT RECOMMENDATION			
KIT	NUMBER OF SAMPLES	NUMBER OF SCROLLS INPUT PER SAMPLE	RECOMMENDED THICKNESS (µm)
Mini	1-12	1	25-50
WT	1-4	2	25-50
	>4	1	25
Mega	1	6	25-50
	2-5	4	25
	6-9	2	25
	≥10	1	25

CRITICAL! We strongly recommend performing an RNA quality assessment to ensure a DV200 of at least 30%. See Important Guidelines for more information.

Note: These recommendations are based on historical experimental data indicating average yields of ~300,000 nuclei from 25 µm scrolls and ~600,000-900,000 nuclei from 50 µm scrolls and assuming tissue dimensions of ~10 mm × 5 mm. Ideal input should be determined empirically for each sample, as the total nuclei yield varies across block processing and tissue types. After dissociation and dilution, each stored reaction can hold up to 600,000 dissociated nuclei for storage. Use this value to determine the maximum number of nuclei your kit can support after dissociation.

3. Cut the required number of FFPE scroll sections as determined by the above table from each FFPE block using your preferred device.

4. Transfer a max of two 25 µm or one 50 µm sections from each tissue block into each separate 1.5 mL Eppendorf tube. If processing more than two 25 µm scrolls per sample, split the sample into multiple dissociation reactions.

Note: Adding more than the recommended amount of scrolls to a single 1.5 mL tube increases paraffin carryover, making downstream processing more difficult.

CRITICAL! From step 5 through 19, perform all steps inside a certified chemical fume hood.

5. In a fume hood, add **1 mL** of xylene to each tube containing scrolls, switching tips between each tube.

6. Incubate at 4°C for **40 minutes** in a thermomixer.

7. Using a new Pasteur pipette for each sample, carefully discard the xylene, ensuring the scrolls are not removed.

8. Repeat steps 5–7 two more times, completing three washes total, each with a **40 minute** incubation.

9. For each tube of FFPE scrolls, prepare **2.2 mL** of freshly made 100% ethanol, and **1.1 mL** each of 95%, 70%, and 50% ethanol.

10. Prepare **1.1 mL** of nuclease-free water per tube of FFPE scrolls and keep on ice.

11. In a new 15 mL tube prepare the Dissociation Buffer as follows. Mix by pipetting 10x and store on ice.

DISSOCIATION BUFFER			
Number of Reactions	1	6	12
Nuclease-free water	1,018 µL	6,108 µL	12,216 µL
TRIS 1M	10.3 µL	61.8 µL	123.6 µL
EDTA 0.5M	2 µL	12 µL	24 µL
N-Lauroylsarcosine sodium salt solution	27.5 µL	165 µL	330 µL
Proteinase K	42.2 µL	253.2 µL	506.4 µL
Total	1,100 µL	6,600 µL	13,200 µL

12. Switching tips between each tube, add **1 mL** of 100% ethanol to each tube containing scrolls.

13. Incubate for **1 minute** at room temperature.

14. Carefully discard the ethanol using a new Pasteur pipette for each sample, ensuring the scrolls remain in the tube.

15. Repeat steps 12-14 once more for a total of two 100% ethanol washes.

16. Add **1 mL of 95% ethanol** and incubate for **1 minute** at room temperature. Carefully discard the ethanol using a clean Pasteur pipette for each sample, while keeping the scrolls in the tube.

17. Add **1 mL of 70% ethanol** and incubate for **1 minute** at room temperature. Carefully discard the ethanol using a clean Pasteur pipette for each sample, while keeping the scrolls in the tube.

18. Add **1 mL of 50% ethanol** and incubate for **1 minute** at room temperature. Carefully discard the ethanol using a clean Pasteur pipette for each sample, while keeping the scrolls in the tube.

19. With a new tip, add **1 mL** of cold nuclease-free water.

20. Incubate for **1 minute** on ice.

21. Using a clean Pasteur pipette for each sample, discard the nuclease-free water carefully, ensuring the scrolls remain in the tube.

22. Switching tips between tubes, add **1 mL** of Dissociation Buffer to each tube.

23. Incubate the tubes overnight (15–18 hours) on a thermomixer at 700 rpm and 4°C.

Note: Do not exceed 18 hours on a pre-cooled 4°C thermomixer.

Section 2: Nuclei Storage and Wash

Following overnight dissociation, nuclei are washed to remove residual debris. The nuclei are then resuspended in Nuclei Storage Master Mix and stored at -80 °C.

1. Gather the following items and handle as indicated below.

ITEM	SOURCE	FORMAT	HANDLING AND STORAGE
Storage Buffer	FFPE Nuclei Preparation (-20°C)	2 mL tube	Thaw at room temperature then immediately store on ice. Mix by inverting each tube/bottle. Do not vortex.
RNase Inhibitor	FFPE Nuclei Preparation (-20°C)	1.5 mL tube	Store on ice immediately before use. Do not vortex.
DMSO	FFPE Nuclei Preparation (-20°C)	1.5 mL tube	Thaw and store at room temperature. Mix by inverting the tube.

2. Cool a centrifuge with swinging bucket rotors to 4°C.
3. Prepare an appropriate cell counting device.
4. Prepare **2.2 mL** of PBS per tube of FFPE scrolls and keep on ice.
5. Prepare the Nuclei Storage Master Mix in a new tube as follows. Mix thoroughly by pipetting and store on ice.

NUCLEI STORAGE MASTER MIX			
Number of Reactions	1	6	12
Storage Buffer	131.2 µL	787.5 µL	1,575 µL
RNase Inhibitor	1.8 µL	10.5 µL	21 µL
DMSO	7 µL	42 µL	84 µL
Total Volume	140 µL	840 µL	1,680 µL

6. Retrieve samples from the thermomixer and store on ice.

7. Using a sterile pestle, gently dissociate any remaining tissue in the tube using light pushes and twisting motions.

Note: If the tissue is more resistant due to residual paraffin, apply firmer pressure as needed to help break the tissue apart.

8. Centrifuge the 1.5 mL tube in a swinging bucket rotor for **10 minutes** at 10,000 x g at 4°C. Immediately move to the next step after centrifugation.

9. Remove the supernatant until about ~40 µL of liquid remains above the pellet. Use a P1000 for the first 500 µL and then a P200 for the remaining volume.

10. Fully but gently resuspend the pellet in **1 mL** of pre-cold PBS.

11. Centrifuge the 1.5 mL tube in a swinging bucket rotor for **10 minutes** at 10,000 x g at 4°C. Immediately move to the next step after centrifugation.

12. Remove the supernatant until about ~40 µL of liquid remains above the pellet. Use a P1000 for the first 500 µL and then a P200 for the remaining volume.

13. Fully but gently resuspend the pellet in **1 mL** of pre-cold PBS.

14. Pipette the sample(s) through a cell strainer into a new 1.5 mL tube and keep on ice.

15. Centrifuge the 1.5 mL tube in a swinging bucket rotor for **10 minutes** at 300 x g at 4°C (lower speed is intentional as it preferentially pellets strained nuclei). Immediately move to the next step after centrifugation.

16. Remove the supernatant until about ~40 µL of liquid remains above the pellet. Use a P1000 for the first 500 µL and then a P200 for the remaining volume.

17. Resuspend the sample(s) completely in **50 µL** of Nuclei Storage Master Mix.

CRITICAL! Combine any samples split into different dissociation reactions into one tube.

18. While minimizing time on ice, count the number of nuclei in the sample(s) with a fluorescence based counting device and record the total nuclei count.

19. If the sample(s) concentration exceeds 5,000 nuclei/µL after counting, dilute the sample with additional Nuclei Storage Master Mix to bring the concentration to **≤5,000** nuclei/µL.

20. Mix well before each transfer, then evenly distribute the nuclei into PCR strip tubes. Do not exceed 120 µL per tube. At this stage, each tube is considered a "Stored Reaction" and will be referred to as such in the sample loading table.

Note: During downstream processing, FFPE Nuclei Prep kits have a maximum capacity of either 12 reactions or 96 reactions depending on kit size. In order to maximize the output of the kit, split samples evenly into the smallest number of stored reactions possible while staying under the 120 µL per reaction limit. For example if a total sample volume is 180 µL, split into two 90 µL stored reactions.

21. Place the sample(s) in a room temperature styrofoam cooler, close the lid, and store at -80°C to slowly cool the samples.

CRITICAL! Storing samples directly in the freezer without controlled cooling may lead to nuclei damage and compromise data quality.

Safe stopping point: Stored Reaction samples are stable for up to 4 months at -80°C.

Appendix: Revision History

Version	Description	Date
1.0	Initial Release	April 2026
1.1	Updated sample naming convention	April 2026
1.2	Important Guidelines section: Added guidance on recommended RNA integrity	May 2026

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