LeviPrep Nuclei Kit II (16 rxn or 4 rxn Demo)

PRODUCT DATA SHEET

Catalog #1005055 (16 rxn Kit) / #1005056 (4 rxn Demo Kit)
RUO: For Research Use Only. Not for use in diagnostic procedures.

Description

The LeviPrep™ Nuclei Kit II provides reagents and consumables for the complete nuclei extraction and enrichment workflow. The first part of the workflow consists of nuclei extraction from a variety of starting sample types, and the second part consists of debris removal and nuclei enrichment on the LeviCell® 1.0 or LeviCell EOS systems. Nuclei extraction is performed in Nuclei Extraction Buffer (NIB) using Buffer N3 with a standard tube and pestle format. The protocol has been optimized using brain tissue with amounts ranging from 20 mg to 50 mg per reaction. Larger amounts of brain require scale-up according to the mass of tissue used. Less dense tissues such as lung may require larger input quantities and optimization per sample type. Extraction is followed by a large volume wash step. Nuclei extraction and enrichment on the LeviCell systems using the LeviPrep Nuclei Kit II is optimized for removal of ambient nucleic acid, debris, and nuclei clumps from the nuclei sample.

Kit Components

Reagents	PN	Quantity (16 rxn)	Quantity (4 rxn)	Shipping Conditions	Storage Conditions	Prep for use
10X Buffer N3	6000115	1 x 6 mL	1 x 6 mL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component L	6000110	1 x 600 µL	1 x 600 µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component A[1]	6000109	2 x 1400 µL	1 x 1400 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw at RT and place on ice
Component R[1]	6000114	1 x 350 µL	1 x 350 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw on ice
Particle M	6000106	4 x 55 µL	1 x 55 µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Equilibrate to RT for use
2X Buffer WB	6000101	1 x 75 mL	1 x 75 mL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice

[1] Components A and R need to be transferred to -20°C immediately upon arrival. Components A and R are not stable with long term storage at 2-8°C .

Consumables	PN	Quantity (16 rxn)	Quantity (4 rxn)	Shipping Conditions	Storage Conditions
1.5 mL tubes (sterile/RNase free)	6000077	16 ea	4 ea	Ambient Temperature	Store at room temperature
Pestles (sterile/RNase Free)	6000077	16 ea	4 ea	Ambient Temperature	Store at room temperature
40 µm Filters	6000102	16 ea	4 ea	Ambient Temperature	Store at room temperature

Additional Reagents, Consumables, and Equipment Required

Reagents

Levitation Agent; LevitasBio® PN 1003001 / 1003002
RNaseOUT™ (Invitrogen™ PN 10777019) or an RNase Inhibitor of choice at 40 U/uL
10% BSA solution (Millipore-Sigma™ PN A1595 or PN 126615)
Nuclease-Free reagent grade water (Invitrogen™ PN 10977-015)
7-AAD (Biotium PN 40017 or Propidium Iodide (BD Pharmingen™ PN 559925) stain (optional)

Consumables

LeviCell S2.3 Cartridge; LevitasBio PN 1002010
OR LeviCell EOS-4 Cartridge; LevitasBio PN 1002101
1.5 -2.0 mL low-bind microcentrifuge tubes
50 mL conical tubes
0.1-2 µL, 2-20µL, 20-200 µL, and 200-1000 µL tips
Dissection materials (disposable scalpel, petri dish,etc.)
5 mL tubes (optional)

Equipment

LeviCell 1.0 System; LevitasBio PN 1000001
OR LeviCell EOS System; LevitasBio PN 1000021
0.1-2 µL, 2-20µL, 20-200 µL, and 200-1000 µL pipettes
Dissection tools (scissors, forceps etc.)
Centrifuge (preferably a refrigerated centrifuge with a swinging bucket rotor)
Vortexer
Cell Counter (Fluorescence compatible counter is recommended)

LeviPrep Nuclei Kit II Protocol

Prior to beginning the nuclei extraction, prepare and store all the necessary buffers on ice, or as instructed. To minimize the amount of time the sample is kept on ice or at ambient temperature, keep the tissue sample in storage until after all buffers have been prepared.

1. Prepare individual buffers for the number of samples that can be processed in a single day by following the respective buffer tables.
2. Ensure buffer tubes are labeled according to buffer name.
3. Store on ice throughout the protocol.
4. Mix via inversion or by pipetting after all components have been added.

Prepare Nuclei Isolation Buffer (NIB) - *Store on Ice*

- ***NIB*** should be used in the same day it was prepared.
- Add the volumes listed in Table 1 in the order shown.
- For more than 3 samples, a 5 ml tube is recommended.

[2] Aliquot Component A into smaller volumes to avoid more than two freeze-thaw cycles.

[3] RNaseOUT is not supplied with the kit and can be substituted with the RNase Inhibitor of the user's choice. Final RNase Inhibitor concentration should be at 0.2 U/µL.

Table 1. Volumes to prepare ***NIB***

Reagent (Vol. in µl)	Volume for 1 sample plus overage	Volume for 4 samples plus overage
10X Buffer N3	50.6	202
Nuclease-free water	455	1822
Component L	6	24
Component R	3	12
Component A[2]	58	232
RNaseOUT[3]	3	12
Total	576	2304

Prepare 1X Buffer WBB - *Store on Ice*

- ***1X Buffer WBB*** is stable for up to 1 week on ice or at 4°C storage conditions.

Table 2. Volumes to prepare ***1X Buffer WBB***

Reagent (Vol. in ml)	Volume for 1 sample plus overage	Volume for 4 samples plus overage
2X Buffer WB	3.5	14
10% BSA	0.7	2.8
Nuclease Free Water	2.8	11.2
Total	7	28

Prepare NucRes - *Store on Ice*

- ***NucRes*** is stable for up to 8 hours on ice.

Table 3. Volumes to prepare ***NucRes***

Reagent (Vol. in µl)	Volume for 1 sample plus overage	Volume for 4 samples plus overage
1X Buffer WBB	147	588
Component R	1.5	6
RNaseOUT[4]	1.5	6
Total	150	600

[4] RNaseOUT is not supplied with the kit and can be substituted with the RNase Inhibitor of the user's choice. Final RNase Inhibitor concentration in NucRes should be at 0.4 U/µL.

Prepare NucLev - *Store at Room Temperature*

1. Remove the Particle M reagent from the refridgerator and bring to room temperature prior to use.
2. Vortex the tube of Particle M, approx. 30 secs. Flick tube several times to ensure the particles are completely dispersed and homogenous.
3. Per amount tissue, use Table 5 to prepare ***NucLev*** reagent.
4. Pipet up and down prior to adding to the ***NucLev*** reagent.

- Volumes for Table 4 provided are based on 1 sample for specified mass. Multiply by number of samples to make enough ***NucLev*** for all samples processed.
- If using more than 50mg of tissue divide sample into two.
- ***NucLev*** is stable for up to 8 hours on ice.

Table 4. Volumes used to make ***NucLev*** for 1 sample

Reagent (Vol. in µl)	Tissue amount			
	20 mg	30 mg	40 mg	50 mg
1xBuffer WBB[a]	100	97.5	95	92.5
Levitation Agent	45	45	45	45
Particle M	5	7.5	10	12.5
Total	150	150	150	150

[a] If desired, include a dye compatible with the downstream workflow, such as 7-AAD, in this volume. If using 7-AAD, double the manufacturer's recommended dye to cell ratio and reduce the volume of NucRes to accommodate the volume of dye. Note that 7-AAD may not be visible on the LeviCell with low numbers of nuclei.

Fresh/Fresh-Frozen Tissue Sample Tips

- Before starting, ensure the workbench, pipettes, and gloves are RNase-free.
- Larger chunks of tissue work better than finely minced tissue (20 mg up to 50 mg in weight). Cutting the tissue into small pieces (5 mm^3) instead of mincing it will reduce the amount of debris that is generated in the nuclei extraction process.
- This kit has been validated on brain tissue. For some tissues with a lower cell/mass ratio, the amount of starting material may need to be optimized to achieve the expected results.
- When homogenizing soft tissues (e.g., brain, liver, breast), go straight up and down and try not to twist or grind the sample. This will reduce the amount of nuclei clumps that are formed.
- For tougher organs, or tissue with significant amounts of calcification or connective tissue (e.g., muscle, heart, kidney) the pestle can be twisted by a quarter turn to aid in the release of nuclei.
- When mixing, set the pipette to at least half the volume of the sample and pipette mix 1-2 times per second for a total of 5-10 times for best results. Avoid vortexing or aggressive mixing.
- During the tissue homogenization, Step 4 of Nuclei Extraction), a small aliquot of the supernatant should be removed. Dilute with an appropriate volume of propidium iodide (PI)-containing buffer per vendor recommended protocol, and count. If the nuclei yield is low, additional homogenization with the pestle and longer incubation time in the NIB may help to increase yield.

Figure 1. *Frozen mouse brain is diced into small pieces of ~5mm^3 prior to placement into a microtube homogenizer (provided).*

Figure 2. *(Pictured on Left) Small tissue aggregates and particles remain post-homogenization. This is normal, do not over homogenize.*

Figure 3. *(Pictured on Right) Homogenate is passed through the pre-wet 40 µm sieve and washed with 4 mL of 1X Buffer WB. Some retentate on the sieve surface is expected.*

Nuclei Extraction

1. Weigh and place small piece(s) (20 mg - 50 mg) of thawed tissue (fresh or previously frozen) in a 1.5 mL tube provided.
2. Pipet 500 µL of the ***NIB*** into the tube containing the tissue.
3. Homogenize tissue with the pestle, 10-15X.

   ! CAUTION: Depending on the tissue type, some tissue may remain intact (visible clumps); this is normal. Do not over homogenize, as this could decrease nuclei quality.
4. Incubate the homogenized tissue on ice for 5 min.
5. Pre-wet the 40 µm strainer with 500 µL of ***1X Buffer WBB*** into a labeled 50 mL conical tube.

   *! Note: RNase Inhibitor (such as RNaseOUT, can be added to 1X **Buffer WBB** at a final concentration of 0.2 U/uL if desired. A side-by-side comparison withholding versus including RNase Inhibitor from the wash step showed no difference in single-cell sequencing results/metrics.*
6. Pipet the entire sample through the 40 µm strainer into the 50 mL tube.
7. Rinse the 1.5 mL tube and strainer with 4 mL of ice-cold ***1X Buffer WBB***. Total volume should be 5 mL.
8. Centrifuge the sample at 4°C, 500 X g for 3 min.

   Optional: Transfer the entire volume to a 5 ml Eppendorf-style tube prior to this centrifugation step to aid in nuclei pellet recovery.
9. Remove supernatant and resuspend the nuclei in 2 mL of ice-cold ***1X Buffer WBB***. Transfer to 2 mL tube.
10. Pipette mix sample (pipette up and down 1 to 2 times/second) 5-10X until pellet is dispersed and homogenous.
11. Pellet nuclei by centrifugation at 4°C, 500 X g for 3 min.

    Time-saving step: During this spin, prepare LeviCell 1.0 or LeviCell EOS for loading. Refer to Table 5 for the appropriate instrument parameters.
12. Remove supernatant and resuspend in 125 µL of ***NucRes.***
13. Pipette mix sample (pipette up and down 1 to 2 times/second) 5-10X until pellet is dispersed and homogenous.

    ! Note: Keep the nuclei sample on ice until ready for subsequent levitation preparation step #15 below.
14. Sample is ready to be counted and further purified on the LeviCell.

    ! Note: Although optional, counting and assessing a small aliquot of the nuclei sample with PI or other nuclear-staining dye on a fluorescent counter or under a microscope can be a useful QC step. To minimize protocol time, this QC may be performed during or after the LeviCell run.

Nuclei Enrichment

15. Pipette 120 µL of nuclei obtained in step 12 into a fresh 1.5 mL microcentrifuge tube. If less than 120 µL of nuclei sample is being used to conserve sample for other processes, add additional ***NucRes*** to bring sample volume up to 120 µL.

! Note: Optimal results are obtained by loading a volume of nuclei equivalent to 10-50 mg of tissue. Overloading the LeviCell cartridge separation channel may lead to unexpected results.

! Note: Steps 15 on should be performed at room temperature but should not take more than 5'.

16. Add 120 µl of well-mixed ***NucLev*** to the nuclei, and homogenize by gentle pipette mixing approximately 10 times. This is the levitation input sample. Label this tube with a unique sample name. Immediately load the sample into an initialized in the LeviCell instrument, following the software instructions.

⚠ *Caution: Ensure **NucLev** is homogenous and well mixed prior to addition. Pipette samples carefully as there is only a 20 uL overage that is easily lost when using a P1000 pipette. Leftover sample can be used for input sample analysis, if desired.*

Running the LeviCell 1.0 or LeviCell EOS Systems

Follow the instructions per LeviCell 1.0 or LeviCell EOS user guide for instrument setup instructions using the parameters listed in Table 5.

Table 5. Parameters for instrument setup using either the LeviCell 1.0 or LeviCell EOS.

! Note: If additional Levitation time is desired, allow the LeviCell 1.0 or LeviCell EOS to continue into count-up mode. Nuclei have been tested in the LeviCell systems for up to 40 minutes.

Parameter	Setting
Protocol	Nuclei
Levitation Agent Concentration	150 mM
Fluorescent Imaging	Optional
Split Line	0
Volume to Load	220 µL
Sample Collection	Bottom Well

17. Collect purified nuclei from the *BOTTOM* wells of the EOS-4 (Fig 4B) or the LeviCell S2.3 cartridge (Fig 4C).

! Caution: Do not discard the flowthrough from the BOTTOM channel, this is the enriched nuclei sample. The TOP channel flowthrough may be discarded.

*! **LeviCell 1.0 users** ! Be certain to collect all of the volume from the BOTTOM channel by pipetting from the 3 locations indicated on screen. The volume obtained from the bottom channel should be ~80 µL.*

18. Count and QC nuclei using fluorescent cell counter and/or microscopy (Fig 4 D,E). If dye was added to the NucLev, the sample may need to be re-stained for counting/QC.

! Note: The output sample should be diluted 5-fold for counting.

19. The nuclei prep is ready to proceed to downstream workflows such as single nuclei sequencing or proteomic analysis.

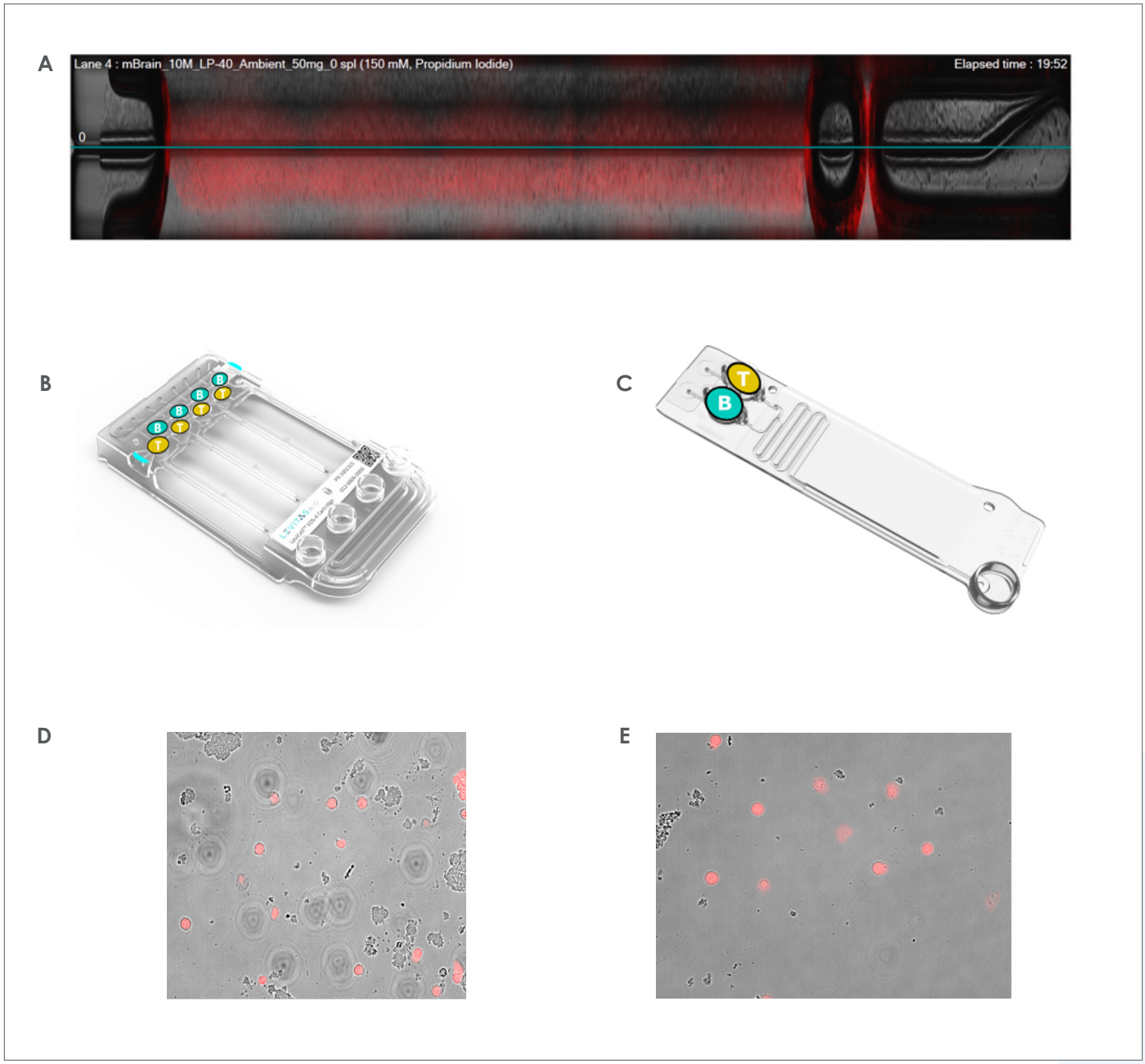

Figure 4. *(A) PI-stained nuclei equilibrate to the bottom channel of a single lane of a LeviCell EOS-4 cartridge. Fluorescently-labeled and unlabeled debris, and nuclei aggregates are removed. A split line of 0 is selected (blue)*

(B, C) Collect nuclei from the bottom well of the EOS-4 (B) or S2.3 cartridge (C). Nuclei should be harvested from the wells labeled "B" (teal). The flowthrough in the well labeled "T" (orange) may be discarded.

(D, E) 20X image of PI-stained brain nuclei pre-levitation (D) and post-levitation (E) after extraction with the LeviPrep Nuclei Kit II protocol.