LeviPrep Nuclei Kit (16 Rxn or 4 Rxn Demo)

PRODUCT DATA SHEET

Catalog# 1005050/1005051

RUO: For Research Use Only. Not for use in diagnostic procedures.

Description

The LeviPrep™ Nuclei Kit 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 Isolation Buffer (NIB) using Buffer N3 with a standard tube and pestle format. The protocol has been optimized using brain tissue ranging from 10 mg to 100 mg in mass. Larger amounts of brain and similar tissues 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. Enrichment on the LeviCell systems using Particle C 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
Buffer N3	6000091	1 x 8.5 mL	1 x 8.5 mL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component L	6000092	1 x 100 µL	1 x 100 µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Place on ice
Component A[1]	6000093	4 x 240 µL	1 x 240 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw at RT and place on ice
Component R[1]	6000096	1 x 50 µL	1 x 50 µL	Ship at 2-8°C.	**Store at -20°C.** Avoid freeze-thaw cycles	Thaw on ice
Particle C	6000094	4 x 380µL	1 x 380µL	Ship at 2-8°C.	Store at 2-8°C. Do not freeze	Equilibrate to RT for use
Buffer WB	6000095	1 x 115 mL	1 x 115 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
70 µm Filters	6000079	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
7-AAD (optional)

CONSUMABLES

LeviCell S2.3 Cartridge; LevitasBio PN 1002010 /
LeviCell S2.3-IR Cartridge; PN 1002012
OR
LeviCell EOS-4 Cartridge; LevitasBio PN 1002101
1.5 -2.0 mL low-bind microcentrifuge tubes
50 mL conical tubes
5 mL tubes
0.1-2 µL, 2-20µL, 20-200 µL, and 200-1000 µL tips
Dissection materials (disposable scalpel, petri dish, etc.)

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 Reagent Preparation

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

Prepare Nuclei Isolation Buffer (NIB) - Store on Ice

Table 1. Prepare Nuclei Isolation buffer for the number of samples being processed in a single day. For more than 3 samples, a 5 mL tube is recommended. Label this ***"NIB."*** Store on ice throughout the protocol. Once prepared, NIB should be used within the day. Mix via inversion or by pipetting after all components have been added.

Reagent	Volume for 1 sample (µL) overage included	Volume for 4 samples (µL) overage included
Buffer N3	506	2024
Component L	6	24
Component R	3	12
Component A[2]	58	232
RNaseOUT[3]	3	12
Total	576	2304

[2] Component A should be stored at -20°C immediately after arrival. Once thawed (on ice), it can be stored at 2-8°C for up to 72 hrs or refrozen twice.

[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.

Prepare NucRes - Store on Ice

Table 2. Prepare the NucRes Buffer in an appropriately-sized tube. Label this ***"NucRes"***. Once prepared, NucRes is stable for up to 8 hrs on ice. Mix via inversion or by pipetting after all components have been added.

Reagent	Volume for 1 sample (µL) overage included	Volume for 4 samples (µL) overage included
Buffer WB	597	2388
RNaseOUT[4]	3	12
Total	600	2400

[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 should be at 0.2U/uL.

Prepare NucLev - Store at room temperature

! Note: Remove the Particle C reagent from the fridge and bring it to room temperature for use.

*! Note: Vortex the tube of Particle C well, ~30 sec. Flick tube several times to be certain that the particles are completely dispersed and homogenous prior to addition to the **NucLev.***

Table 3. Prepare ***NucLev*** in a 1.5 mL tube and label this ***"NucLev".*** Once prepared, ***NucLev*** is stable for up to 8 hrs at room temperature. Pipette NucLev well after all components are added.

Reagent	Volume for 1 sample (µL) overage included	Volume for 4 samples (µL) overage included
NucRes	33*	132
Levitation Agent	47	188
Particle C **(Mix well prior to use)**	92	368
Total	172	688

* *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 (10 mg up to 100 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 isolation process.
- This kit has been validated on brain tissue. For some tissues with lower cell/mass ratio, the amount of starting material may need to be optimized to achieve 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.

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

Nuclei Isolation

1. Weigh and place small piece(s) (10 mg - 100 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, but no more than 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.

Figure 2. *(Left) Small tissue aggregates and particles remain post-homogenization. This is normal, do not over homogenize. (Right) Homogenate is passed through the sieve and washed with 4 mL of Buffer WB. Some retentate on the sieve surface is expected.*

4. Incubate the homogenized tissue on ice for 5 min.

5. Prewet the 70 µm strainer with 500 µL of ***Buffer WB*** into a labeled 50 mL conical tube.

   *! Note: RNase Inhibitor (such as RNaseOUT, can be added to **Buffer WB** 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 70 µm strainer into the 50 mL tube.

7. Rinse the 1.5ml tube and strainer with 4 mL of ice-cold ***Buffer WB***. Total volume should be 5 mL.

   ! Optional: The volume may be transferred to a 5ml tube if desired.

8. Centrifuge the sample at 4°C, 500 X g for 3 min.

9. Remove supernatant and resuspend the nuclei in 2 mL of ice-cold ***Buffer WB***. Transfer to 2 mL tube. Pipette mix sample (pipette up and down 1 to 2 times/second) 5-10X until pellet is dispersed and homogenous.

10. Pellet nuclei by centrifugation at 4°C, 500 X g for 3 min.

    Time saving step: During this spin, prepare LeviCell 1.0 or the LeviCell EOS system for loading. Refer to Table 5 for the appropriate instrument parameters.

11. Remove supernatant and resuspend in 150 µL of ***NucRes***.

12. 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.

13. 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 7-AAD or other 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 Levitation and Cleanup

14. Pipette 125 µL of nuclei obtained in step 12 into a fresh 1.5 mL microcentrifuge tube. If less than 125 µL of nuclei sample is being used to conserve sample for other processes, add additional ***NucRes*** to bring sample volume up to 125 µL.

    ! Note: Optimal results are obtained by loading a volume of nuclei equivalent to 10-100 mg of tissue. Overloading the LeviCell cartridge separation channel may lead to unexpected results.

15. Add 150 µl of well-mixed ***NucLev*** to the nuclei, 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 LeviCell 1.0 or the LeviCell EOS system

    ⚠ Caution: Ensure NucLev is homogenous and well-mixed prior to addition.

Running the LeviCell 1.0 or LeviCell EOS Systems

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

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

Parameters	LeviCell 1.0	LeviCell EOS
Protocol	Nuclei	Nuclei
Levitation Agent Concentration	150 mM	150 mM
Fluorescent Imaging	Optional	Optional
Split Line	-15 (For AutoRun, enter split line during experiment setup)	-15 (For AutoRun, set split line and click on "Split line confirmed for collection")
Volume to Load	220 µL	220 µL
Sample Collection	Bottom Well	Bottom Well

! Note: Particle C will cause the screen to look completely black at the beginning of the run; this is normal. The particles will begin to resolve within 2 min of starting the run.

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

! Nuclei are found in the bottom channel of the levitation run. A split line of -15 is strongly suggested.(Fig 3) Deviation from a split line setting of -15 may lead to unexpected results.

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

    ! 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 ~40 µL.

17. Count and QC nuclei using fluorescent cell counter and/or microscopy (Fig 3). 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.

    ! Some carry over of clean-up particles is normal and will not negatively affect downstream workflows.

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

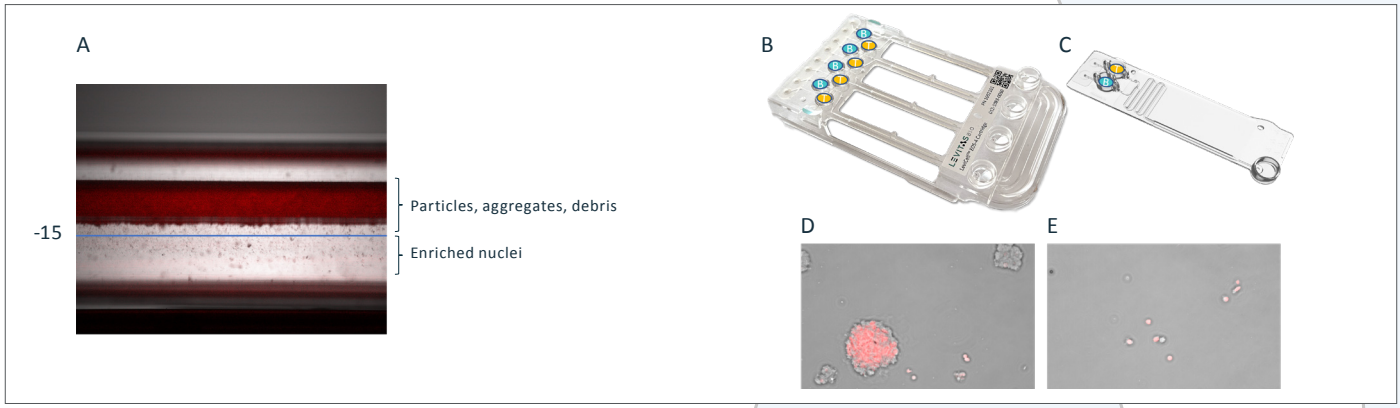

Figure 3. *(A) 7-AAD stained nuclei equilibrate to the bottom channel of the LeviCell cartridge. Fluorescently labeled debris, nuclei aggregates and particles are removed. A split line of -15 is selected (blue) B,C) Collect nuclei from the EOS-4 (B) or LeviCell s2.3 cartridge (C). Nuclei should be harvested from the wells labeled "B" - teal. The flowthrough in the well labeled "T" – orange may be discarded. (D,E) 20X image of 7-AAD stained nuclei pre(C) and post (D) LeviPrep Nuclei Kit protocol.*