LeviSelect Cell Debris Depletion Kit (96 rxn)

PRODUCT DATA SHEET

Catalog# 1004062

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

Description

The LeviSelect™ Cell Debris Depletion Kit is designed to efficiently remove debris from cell suspensions during viable cell enrichment on a LeviCell® 96 instrument. During processing, the majority of debris locates to the walls of the LeviCell 96 sample input plate, while viable, untouched cells are simultaneously separated from dead cells. These enriched, healthy cells are then collected in the output plate. The kit is compatible with a variety of sample types, including fresh, frozen and fixed. While live/dead cell separation is not possible for fixed samples, debris will still be depleted. This data sheet includes protocol options for both sample types.

Kit Components

Component	Tube PN	Quantity	Storage Conditions	Shelf Life
LeviSelect Debris Depletion Nanospheres	6000139	1x 1100 µL	Store at 2-8°C. Do not freeze	Stable until expiration date on label
1x LeviSelect Debris Depletion Buffer	6000141	3x 1.5 mL	Store at 2-8°C. Do not freeze	Stable until expiration date on label

Additional Tools and Consumables Required

LeviCell 96 Complete Enrichment Kit; PN 1002201
1.5 - 2.0 mL low-bind microcentrifuge tubes
2-20 µL, and 200-1000 µL pipettes and tips
Levitation Agent; LevitasBio PN 1003001 or PN 1003007

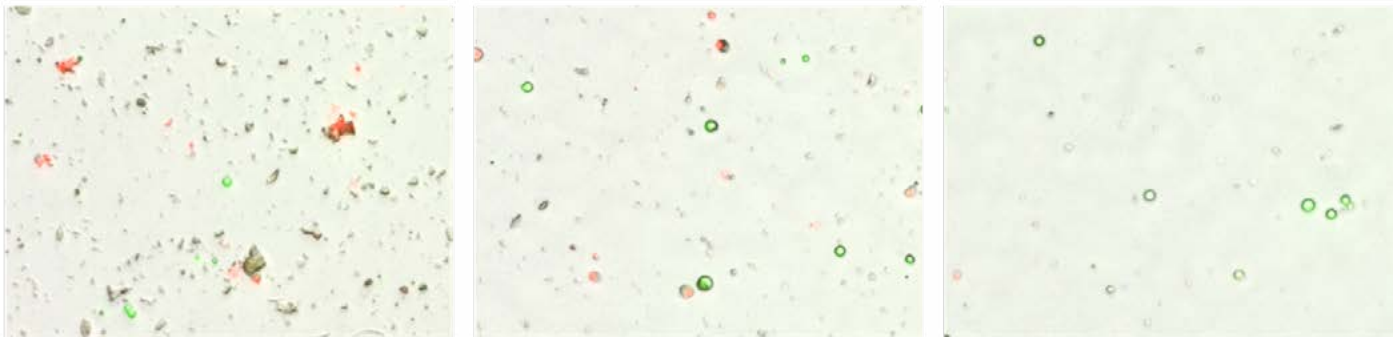
Data example

A) Live Cell Samples

Before Depletion

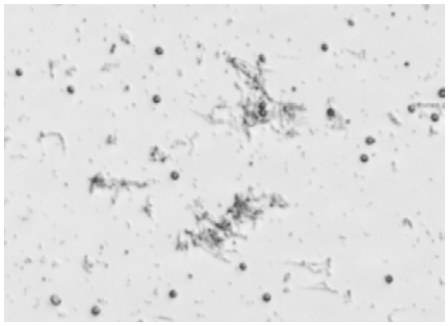
After levitation **without** Cell Debris Depletion

After levitation **with** Cell Debris Depletion



B) Fixed Cell Samples

Before Cell Debris Depletion



After Cell Debris Depletion

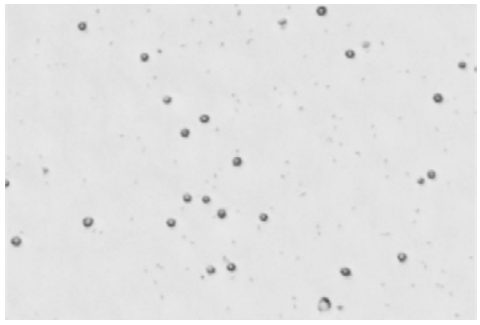
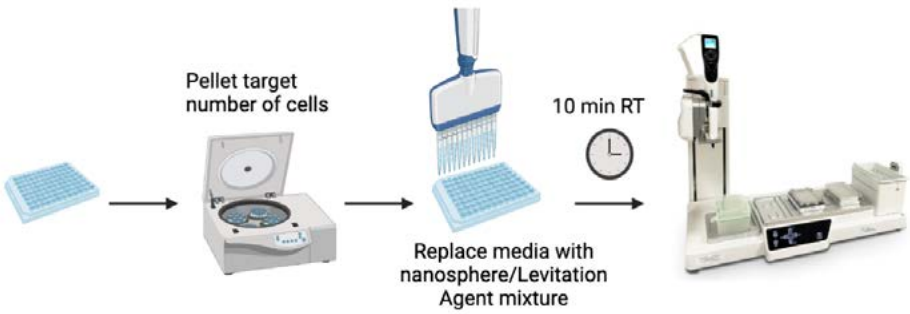


Figure 1. *A) Dissociated human bladder tumor before (left panel), after levitation without debris depletion (middle panel) and after levitation with the LeviSelect Cell Debris Depletion Kit protocol for live cells (right panel). Each picture shows an overlay of brightfield and fluorescence images. Live cells are stained with acridine orange (green) and dead cells are stained with propidium iodide (red). Levitation alone removes a significant portion of debris, but the Cell Debris Depletion Kit removes additional debris. B) Debris-contaminated human lung cancer cells (H358) were fixed in 4% paraformaldehyde and then imaged before (left panel) and after treatment with the LeviSelect Cell Debris Depletion Kit protocol for fixed cells (right panel).*

LeviSelect Cell Debris Depletion Kit Protocol

Workflow



Standard Live Cell Protocol:

Prepare Nanosphere/Levitation Agent Mixture for live cells

Table 1. Standard Live Cell Protocol. *Preparation of the Nanosphere/Levitation Agent Mixture to achieve a concentration of 100 mM Levitation Agent in the final sample. This preparation contains* ***1 µL*** *nanospheres per sample in a total volume of 50 µL per sample.*

Reagent Volume	For 1 sample (µL; includes 10% overage)	For 12 samples (µL; includes 10% overage)	For 96 samples (µL; includes 10% overage)
1X LeviSelect Debris Depletion Buffer	48.4	580.8	4646.4
Levitation Agent	5.5	66	528
LeviSelect Debris Depletion Nanospheres*	1.1	13.2	105.6
Total	55	660	5280

**Vortex the LeviSelect Debris Depletion Nanospheres immediately before use.*

Fixed Cell Protocol:

Prepare Nanosphere/Levitation Agent Mixture for fixed cells

Table 2. Fixed Cell Protocol. *Preparation of the Nanosphere/Levitation Agent Mixture to achieve a concentration of 100 mM Levitation Agent in the final sample. This preparation contains* ***10 µL*** *nanospheres per sample in a total volume of 50 µL per sample.*

Reagent Volume	For 1 sample (µL; includes 10% overage)	For 12 samples (µL; includes 10% overage)	For 96 samples (µL; includes 10% overage)
1X LeviSelect Debris Depletion Buffer	38.5	462	3696
Levitation Agent	5.5	66	528
LeviSelect Debris Depletion Nanospheres*	11	132	1056
Total	55	660	5280

**Vortex the LeviSelect Debris Depletion Nanospheres immediately before use.*

LeviCell 96 Run with Cell Debris Depletion

1. Prepare a cell suspension from tissue/cells of interest. If using cells derived from live tissue, please prepare the nanosphere/levitation agent mixture for live cells in Table 1. If using cells derived from fixed tissue, please prepare the nanosphere/levitation agent mixture for fixed cells in Table 2.
2. Count cell suspensions for both viability (if starting with live cells) and cell concentration (both live and fixed cells). If possible, take images in both the brightfield channel as well as using nuclei-specific fluorescent dyes to detect live and dead nucleated cells, for example, a combination of acridine orange and propidium iodide (AOPI). Depending on the type of debris, distinguishing between actual cells and debris can be challenging. Therefore, aliquot no more than 5 x 105 total events based on the brightfield counts from the prepared cell suspension into each well.
3. Centrifuge the cells at 300 RCF for 5 minutes, and then remove the supernatant.
4. Vortex the appropriate Nanosphere/Levitation Agent Mixture and resuspend cell pellets (from step 3) with 50 µL of the mixture in each well. Pipette mix 10 times. ***Be careful not to introduce bubbles.***
5. Seal the plate with the film provided and incubate the plate for 10 minutes at room temperature.
6. Set the sample input plate in the Levitation Array for 40 minutes. Meanwhile, set up a sample output plate with 50-100 µl of the preferred collection buffer added to each output well. Seal the plate and set it in the output block of the LeviCell 96.
7. After levitation, use the appropriate program on the LeviCell 96 to collect your live or fixed cells.
    a. If live, use the **VCE96_C** program to collect the live cells by viable cell enrichment.
    b. If fixed, use **FIXED-AD-48_C** (for rows A-D) and **FIXED-EH-48_C** (for rows E-H) programs to collect the fixed cells.
    c. Follow all instructions on the pipette screen. The desired debris-free cells will be collected and transferred to the wells of the sample output plate.