SARTORIUS

Product Guide

Incucyte® Exofluor EV Labeling Kits

For use in Incucyte® Live-Cell Analysis Systems

Product Information

Presentation, Storage and Stability

The Incucyte® Exofluor EV Labeling Kits are designed to fluorescently label extracellular vesicles (EVs) (e.g., exosomes) in order to investigate uptake in live-cell assays using the Incucyte® Live-Cell Analysis System. Each kit includes lyophilized Incucyte® Exofluor EV Labeling Dye (for labeling EVs), Vivaspin® 2 concentrators (for removing free dye), and Minisart® filters (for sterilization and removal of unbound aggregate dye). Post-processing, labeled EVs are added to cells through a full media replacement, and uptake is measured by quantifying fluorescence using the Incucyte® Live-Cell Analysis System. The EV labeling kits are compatible with EVs isolated using a variety of methods (including precipitation, TFF/IEC, ultrafiltration and ultracentrifugation) and contains components needed for 10 labeling reactions. These kits are intended for use in the Incucyte® CX3, SX5, S3, or SX1 Live-Cell Analysis Systems.

Find out more: www.sartorius.com/incucyte

Product Name	Cat. No.	Ex. Max	Em. Max	Amt.	Storage	Stability
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Red or Green | Orange | Near-IR Optical Module						
Incucyte® Exofluor EV Labeling Kits				1 kit		
▪ 1 vial of Incucyte® Exofluor Green, Red or NIR EV Labeling Dye						
- Incucyte® Exofluor Green EV Labeling Dye	BA-04877	480 nm	513 nm	1 vial	–20 °C	12 months from date of receipt
- Incucyte® Exofluor Red EV Labeling Dye	BA-04900	638 nm	667 nm	1 vial	-20°C	12 months from date of receipt
- Incucyte® Exofluor NIR EV Labeling Dye	BA-04901	638 nm	667 nm	1 vial	–20 °C	12 months from date of receipt
▪ Vivaspin® 2, PES*, 100 kDa				10 units	15 – 30 °C	Please refer to product label
▪ Minisart® PES, 0.22 µm				10 units	15 – 30 °C	Please refer to product label

* PES--Polyethersulfone

Background

Extracellular vesicles (EVs) are heterogeneous, lipid-bound particles (50 – 1000 nm) naturally secreted by cells into the extracellular space. Exosomes are a subclass of EVs defined by both size (40 to 160 nm) and biogenesis (endosomal origin)[1]. Given their role in transporting biomolecules (e.g. proteins, lipids, nucleic acids), EVs are of diagnostic and therapeutic interest to researchers. The Incucyte® Exofluor EV Labeling Kits enable non-perturbing, real-time dynamic evaluation of cellular EV uptake and have been validated for use with the Incucyte® Live-Cell Analysis System configured with a Green | Red or Green | Orange | Near-IR optical module. Incucyte® Exofluor EV Labeling Dye uniformly labels the plasma membrane of small EVs (e.g., exosomes) by covalently binding to proteins and amino acids. The irreversible binding to the EVs, combined with the removal of free dye, mitigates the risk of non-specific dye attachment to other plasma membranes before or during uptake.

Recommended Use

We recommend that the Incucyte® Exofluor Dye is prepared at a stock concentration using anhydrous DMSO (not supplied). The Incucyte® Exofluor Dye is diluted 1:10 using Phosphate Buffer Saline (Ca^{2+}/Mg^{2+} free) to create the working stock solution used in the 30-minute labeling reaction. The volume of EVs for each labeling reaction will need to be determined by the number of assay wells and the concentration of EVs per well. We recommend using EVs within a range of 1 – 4 µg of protein/well or 5×10^7 – 1×10^9 particles/well. These ranges have been validated by testing a wide range of exosome preparations across several methods of purification. Some optimization may be required depending on the quality of exosome preparation. Vivaspin® 2 columns are used to remove the unlabeled dye from the labeled EVs. Each column can process a maximum load of 40 µg protein or 100 µL labeled EV sample. Higher protein loading may result in membrane clogging and exceeding 100 µL labeled EV sample volume will cause an increase in background fluorescence. After the free dye is removed, a Minisart® syringe filter (0.22 µm) is used for sterilization and removal of unbound aggregates.

Reference

1. Raghu Kalluri, Valerie S. LeBleu. **The biology, function, and biomedical applications of exosomes.** *Science* 2020, 367(6478): eaau6977

Example Data

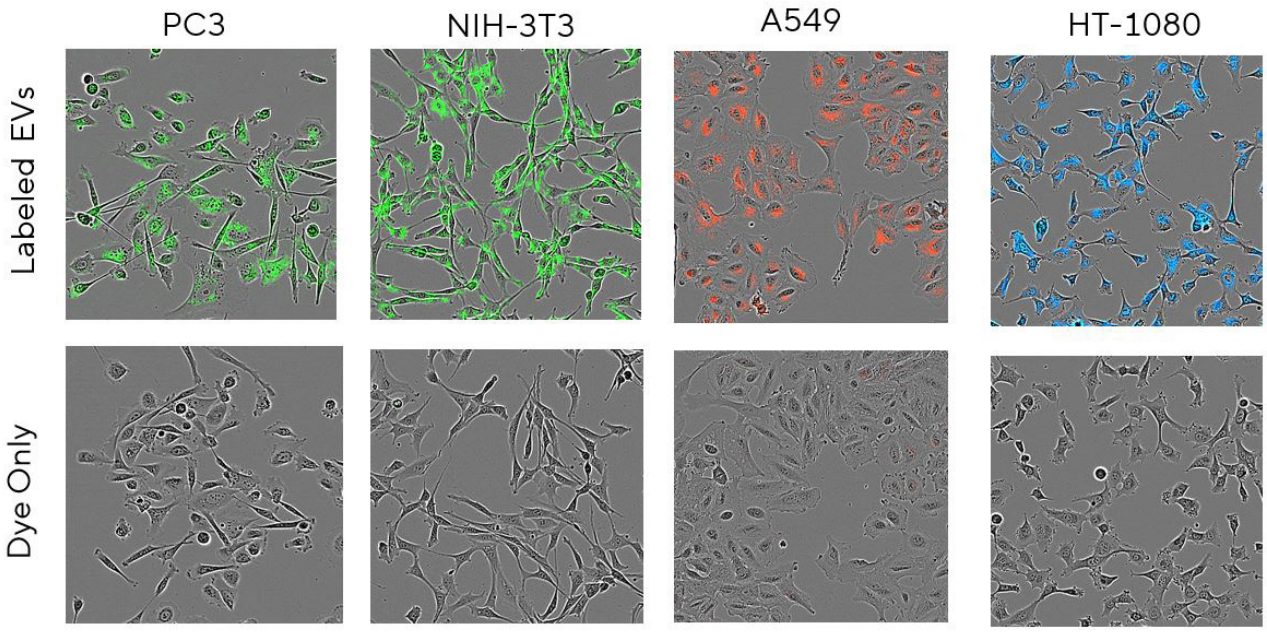


Figure 1: *Uptake Cell Compatibility*

A549 Exosomes (2 µg per well, HansaBioMed) were labeled using the Incucyte® Exofluor Dyes. Uptake of labeled exosomes can be visualized across various cell types and media conditions (top row). Dye only control wells for each condition demonstrate the fluorescent signal is not due to residual free dye (bottom row). Images taken at 24-hours post-treatment with labeled A549-derived exosomes across various cell types show no change in cell morphology compared to controls.

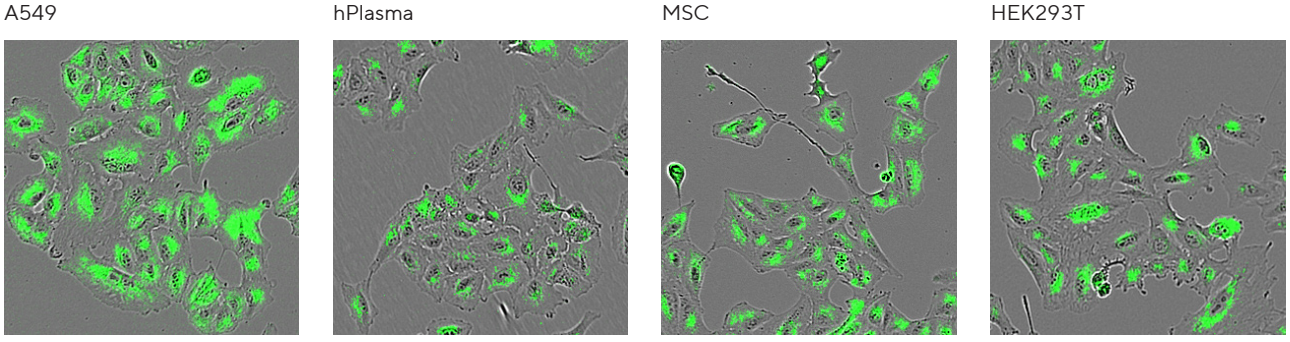


Figure 2: *EV Labeling Compatibility*

Images demonstrate uptake of various EVs labeled with Incucyte® Exofluor Green by A549 recipient cells (24-hours post-treatment). A549 exosomes were extracted using ultrafiltration and SEC methods (HansaBioMed) and treated at 2 µg/well. Human plasma-derived exosomes (Abcam, ultrafiltration method of extraction) were used at a concentration of 4 µg/well. Both MSC and HEK293T EVs (Sartorius) were extracted using TFF/IEX chromatography and treated at 1.5×10^7 and 8.3×10^7 particles/well, respectively.

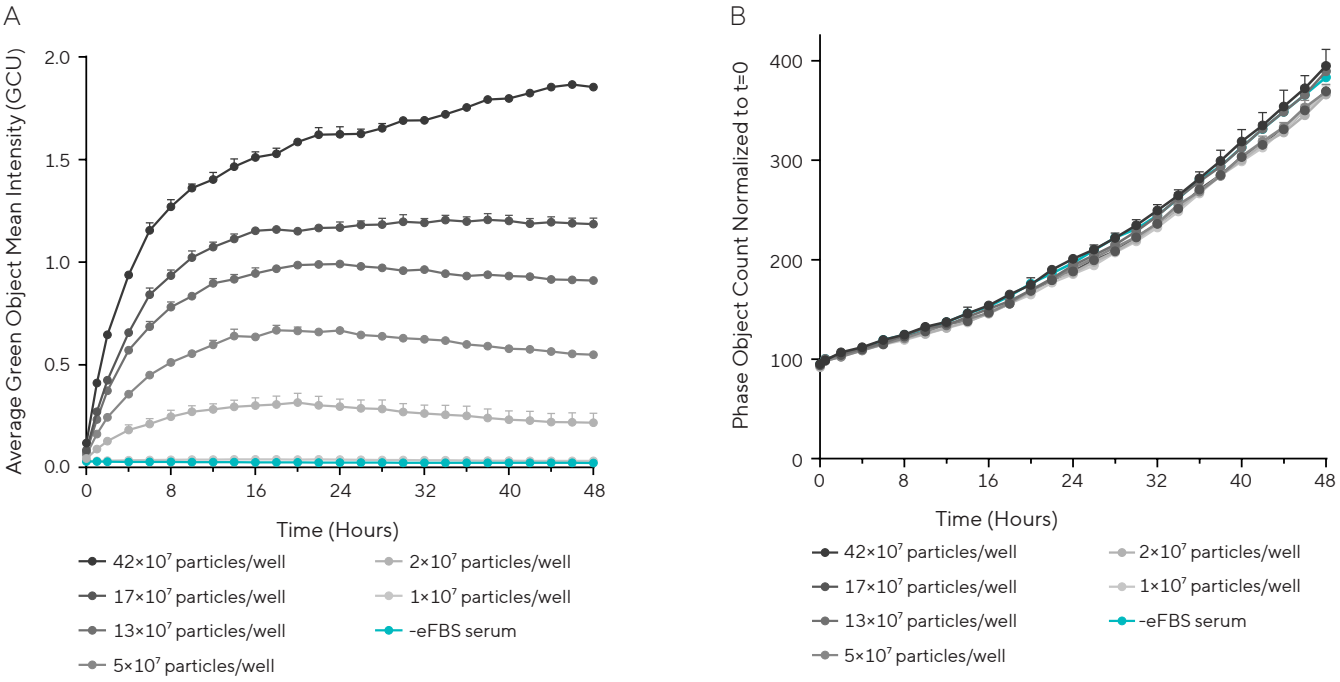


Figure 3: *EV Concentration Dependence*

EVs were purified from HEK293T cells using TFF/IEX chromatography and labeled using the Incucyte® Exofluor Green EV Labeling Kit and added to A549 recipient cells. Phase and green fluorescent images were acquired every 2 hours over the course of 2 days and analyzed using Incucyte® Cell-by-Cell Software. (A) Labeled EVs were titrated ~40-fold (42×10^7 and 1×10^7 particles/well) and uptake was assessed using Average Green Mean Intensity. A corresponding concentration-dependent change in fluorescence was observed. (B) Phase Object Count (normalized to t=0) data illustrates no change in proliferation at all concentrations of labeled EVs tested.

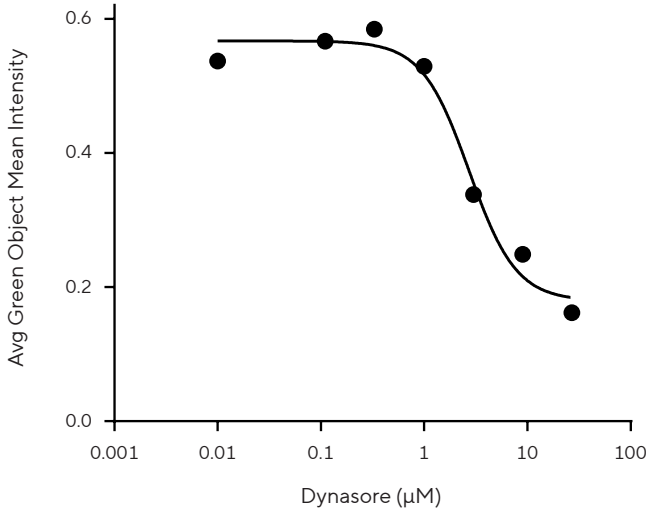
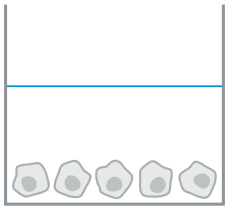


Figure 4: *Exosome Uptake Depends on Endocytosis*

A549 Cells were seeded overnight and then treated with the endocytosis inhibitor Dynasore (0.01 to 100 µM) in exosome-free culture medium. Immediately following Dynasore treatment, Hansa A549 exosomes labeled with Incucyte® Exofluor Green (2 µg/well) were added. A concentration-dependent inhibition (IC_{50} = 2.72 µM) of exosome uptake was reflected in the intensity of green fluorescence (24-hour data shown).

Quick Guide

1. Cell Seeding



Seed cells in growth media and leave to adhere (4 – 24 hours). Cells should be 15 – 35% confluent at the time of treatment.

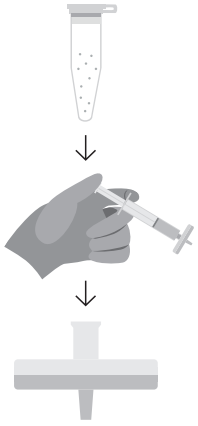
2. EV Labeling

Reconstitute Incucyte® Exofluor EV Labeling Dye in DMSO and add to EVs in a 1:5 -1:8 (v/v) ratio. Incubate labeling reaction for 30 minutes at 37 °C.

3. Free Dye Removal

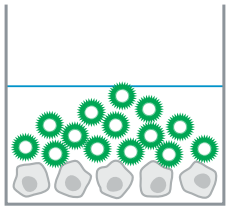
Add EV labeling reaction to the Vivaspin® 2 column (max load 40 µg or 100 µL per column) and spin at 500 x g for 5 minutes to remove free dye.

4. Post-labeling Clean Up



Collect labeled EVs from the Vivaspin® 2 in Assay Medium and load into a syringe. Pass mix through a Minisart® filter (0.22 µm) to sterilize and remove aggregates.

5. Treatment of Cells with Labeled EVs



Perform a full media replacement with labeled EV containing media.

Protocol and Procedures

Materials

- Incucyte® Exofluor EV Labeling Kit (Sartorius Cat. Nos. BA-04877, BA-04900, BA-04901)
- DMSO, anhydrous (e.g. Thermo, Cat. No. D12345)
- PBS (Ca^{2+}/Mg^{2+} free)
- Cell Culture Medium for seeding cells, cell specific
- Exosome-depleted FBS (recommended Gibco, Cat. No. A2720803)
- 96-well microplate, sterile (e.g., Corning® Cat. No. 3595)
- Syringes, sterile (3 – 10 mL)
- (Optional) Gel loading tip, (e.g. PR1MA, Cat. No. PR-200XLRK-FL)
- 16-gauge needle, blunt end needle preferred (e.g. Stemcell Cat. No. 28110)
- Sterile microfuge tubes (preferably amber) for labeling and collection
- Centrifuge (swing bucket or fixed angle) to fit 15 mL conical bottom tube

General Guidelines

Uptake Cell Plates

- Depending on the growth kinetics of your cells, you may need to optimize the seeding density in your assay plate to achieve the desired confluence following overnight incubation. We have found that plating between 1,000 to 5,000 cells per well (10,000 to 50,000 cells/mL) for a 96-well plate is a reasonable starting point.
- Following cell seeding, place plates at ambient temperature to ensure homogenous cell settling (15 – 30 min).
- Remove bubbles from all wells by gently squeezing a wash bottle (containing 70 – 100% ethanol with the inner straw removed) to blow vapor over the surface of each well.

Labeling Protocol

- Allow Incucyte® Exofluor Dye to reach room temperature prior to use and protect from light.
- Recommended controls include Incucyte® Exofluor Dye only control (no EVs) and EV only (dye-free) conditions. **Note:** Controls are prepared at Labeling step (substituting PBS for Dye or EVs) and undergo Free Dye Removal and Post-Labeling Clean-up steps.
- All Vivaspin® 2 centrifugation steps are performed at 500 x g, 5 minutes (room temperature) unless otherwise noted.
- Vivaspin® 2 columns can be centrifuged using a swinging bucket or fixed angle rotor. When a fixed angle rotor is used, it is advisable to angle the column so that the printed window faces upwards | outwards.
- Once a Vivaspin® 2 column has been wetted, do not allow the membrane to dry out.
- For the post-labeling cleanup, we recommend using a blunt end 16-gauge needle for ease of use. A standard 16-gauge (or wider diameter) needle may also be used with added safety measures.
- For optimal assay results, it is advisable to conduct preliminary experiments to determine the optimal EV concentration as this can vary based on the isolation and purification method used. A typical range of EVs in the assay is 1 – 4 µg/well or 5×10^7 – 1×10^9 particles per well.
- A good practice is to label EVs in a single reaction and serial dilute to achieve the recommended or desired range.
- The EV uptake assay is performed using Assay Medium (prepared using exosome-depleted serum, -eFBS) to minimize the presence of endogenous exosomes in FBS affecting uptake kinetics.
- We recommend filtering the PBS and Assay Medium through a 0.22 µm filter to remove any unwanted particulates prior to use.

Example Optimization Plate Map

All	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			Exofluor Green EVs 4 µg/well A549 3K/well									
C			Exofluor Green EVs 2 µg/well A549 3K/well									
D			Exofluor Green EVs1 µg/well A549 3K/well									
E			A549 3K/well Exofluor Green Dye Only									
F			A549 3K/well Assay Medium (-eFBS)									
G			A549 3K/well Standard Growth Medium									
H												

Example Protocol

Day 0

Seed Uptake Cell Plate

1. Prepare cell seeding stock at an appropriate density to achieve 15 – 35% confluency at the time of assay.
2. Seed cells into vessel of choice (e.g. 100 µL per well for a 96-well flat bottom microplate).
3. Allow cells to adhere overnight at 37 °C.

Day 1

EV Labeling Protocol

1. Briefly centrifuge Incucyte® Exofluor Dye to ensure contents are collected at the bottom of the vial.
2. Prepare Incucyte® Exofluor Dye to a final concentration of 5 mM.
   2.1 Add 20 µL of anhydrous DMSO to reconstitute lyophilized Incucyte® Exofluor Dye. Vortex to mix.
   2.2 Briefly centrifuge vial to collect solubilized dye at the bottom of the vial.
3. Determine the number of labeling reactions needed based on the concentration of EVs being tested and the number of wells per plate. Note that there is some volume recovery loss during processing that should be factored into the calculations (15 – 25% depending on volume). See Example Calculation for EV Labeling Box (Steps 1 & 2).
4. Calculate the volume of Incucyte® Exofluor Dye (working stock) needed for each labeling reaction. Ratio of EV to Dye (v/v) may need to be optimized. We recommend working in range of 1:5 to 1:8. (Example Calculation Box Step 3)
5. Prepare a working stock of Incucyte® Exofluor Dye
   5.1 Dilute the Incucyte® Exofluor Dye stock 1:10 using PBS (Ca^{2+}/Mg^{2+} free).
   5.2 Aliquot unneeded Incucyte® Exofluor Dye and store at –20 °C (protected from light) for up to 1-month.
6. Transfer the calculated EV labeling volume to a sterile microfuge tube and protect from light.
   Note: Use PBS for the Dye Only Control if one is included in the experiment.
7. Add calculated volume of Incucyte® Exofluor Dye to EVs to the selected dye to EV ratio. Protect from light.
8. Briefly centrifuge and incubate at 37 °C for 30 minutes.
   Note: Proceed to "Free Dye Removal" steps below during labeling reaction.
9. Stop labeling reaction by adding 500 µL PBS.
10. Keep labeled EVs at room temperature, protected from light until use in Free Dye Removal protocol.

Example Calculation for EV Labeling with the Incucyte® Exofluor Green EV Labeling Kit

1. Consider design of experiment: **(example)**
   a. Number of wells in plate (8)
   b. Quantity of EVs needed per well (2 µg)
   c. Scale for dilution series (none)
   d. Factor volume loss during processing (~25%)
2. It is recommended to use 1 – 4 µg per well for a lyophilized standard exosome or 5×10^7 – 1×10^9 particles per well for custom purification EVs (see Example Optimization Protocol).
   Example calculation (EV Labeling Volume):
   8 wells needed at 2 µg/well = 16 µg of EVs needed in labeling reaction. If you have a 1 µg/µL stock of EVs, 16 µg = 16 µL of EVs in the labeling reaction. Account for volume loss during processing: 16 µL → 20 µL (20 µg) of EVs.
3. Calculate the volume of Incucyte® Exofluor Dye needed based on Dye to EV ratio..
   Example calculation:
   4 µL Dye + 20 µL EV (1:6)

Free Dye Removal

1. Determine the number of Vivaspin® 2 columns needed to process each labeling reaction given the following constraint: the EVs loaded to each Vivaspin® 2 should not exceed 100 µL or 40 µg protein.
   1.1 Exceeding the Vivaspin® 2 capacities may compromise the ability of the column to remove free dye.
   1.2 A single labeling reaction may be separated over multiple Vivaspin® 2 columns post-labeling.
2. Calculate and prepare the Vivaspin® 2 Assay Medium containing -eFBS, see table with "Example Calculations for Recovery off Vivaspin® 2 Column".
   2.1 Calculate the volume of Assay Medium required per Vivaspin® 2 such that EVs are recovered in a final working volume (typically in range of 1 – 4 µg/well or 5×10^7 – 1×10^9 particles/well).
   2.2 For a 96-well plate, a minimum of 100 µL per well is recommended.
   2.3 Assay Medium is typically the standard base medium supplemented with -eFBS instead of standard FBS.

3. Prepare the Vivaspin® 2 columns for use.
   3.1 Add 2 mL PBS to the top section of the Vivaspin® 2 column. Centrifuge Vivaspin® 2 column(s) at 500 x g for 5 minutes and decant lower section of column.
   3.2 Add 1 mL PBS to the top section of the Vivaspin® 2 column. Leave the PBS on the membrane to prevent it from drying out while the labeling reaction is occurring.
4. Transfer the contents of the labeling reaction (EV labeling reaction + 500 µL of PBS) to the top section of the Vivaspin® 2 column(s) containing 1 mL of PBS.
5. Centrifuge Vivaspin® 2 column(s) to remove free dye from labeled EVs, decant waste.
6. Add 2 mL PBS to wash labeled EVs on Vivaspin® 2 column(s), centrifuge and decant waste.
7. Recover labeled EVs off the Vivaspin® 2 (s) by adding Assay Medium containing -eFBS to the Vivaspin® 2 columns(s).
   7.1 Prepare a fresh recovery tube (1 – 5 mL) for each Vivaspin® 2 column.
   7.2 If the recovery volume exceeds 1.5 mL, the fresh recovery tube should be used to hold excess volume of assay medium during recovery step. Loading more than 1.5 mL of Assay Media to a Vivaspin® 2 is not recommended due to potential spill over.
   7.3 Add the calculated Assay Medium ("Example Calculations for EV Recovery Step 3") to all Vivaspin® 2 columns prior to proceeding. This step is important for preventing membranes from drying out.
   Note: if there is residual fluid remaining in the Vivaspin after the final wash, adjust the volume of Assay Medium for the recovery.
   7.4 Pipette Assay Medium up-and-down to recover EVs from the Vivaspin® 2 column and transfer media containing EVs to the labeled collection tube.
   7.5 Use a gel loading tip to collect the last 100 – 400 µL of labeled EVs from the Vivaspin® 2 column(s) and transfer to the labeled collection tube.
   Note: An alternate method to pipet recovery of labeled EVs in medium is reverse centrifugation: (1) remove the filtrate tube, (2) invert the column, (3) insert concentrate recovery cap into filtrate tube and (4) centrifuge at 2000 x g for 2 min.

Example Calculations for Exosome Recovery off Vivaspin® 2 Column

1. Consider design of experiment: **(example)**
   a. Labeling reaction (20 µg)
   b. Exosome concentration (2 µg/well)
   c. Assay volume (100 µL/well)
2. Labeling reaction of 20 µg = 1 Vivaspin® 2 column (column capacity is 40 µg)
3. Calculate volume of Assay Medium required for recovery.
   Example calculation:
   Vivaspin® 2 (labeling reaction) contains 20 µg of exosome. Want final recovery concentration at 2 µg exosome per 100 µL well.
   Recovery volume = 20 µg of exosome x 100 µL well/2 µg exosome/well = 1 mL Assay Media

Post-Labeling Clean-up

1. Prepare a sterile collection tube for each labeling reaction (preferably amber).
2. Pre-wet the Minisart® syringe filter(s). Note, this step is not required but will improve volume recovery and may be necessary if the processing volume is less than 1000 µL (≥ 250 µL loss is expected when using a dry syringe filter).
   2.1 Prepare a sterile sample tube with assay medium (≥ 1.0 mL/sample).
   2.2 Fasten a needle to a syringe (3 – 5 mL depending on processing volume).
   2.3 Prime the syringe with void air and then collect ~1000 µL assay medium. Continue drawing up a post-air gap to clear needle of medium.
   2.4 Remove the needle and attach the Minisart® filter (save packaging for temporary storage of filter).
   2.5 Push the collected medium through Minisart® filter and use void air to clear liquid, dispose spent medium.
   2.6 Carefully remove the Minisart® filter and store it in its original packaging until ready for use.
3. Fasten a sterile needle to the syringe to draw up labeled EVs ensuring a pre- and post-air gap to prime and clear needle. The air gaps should equal 20 – 30% the volume of labeled EVs.
4. After syringe is loaded with media containing EVs, remove needle and attach the 0.22 µm Minisart® filter (use filter from step 2 if prewetting was performed).
5. Slowly push labeled EVs across the Minisart® filter such that flow is drop by drop (e.g. 1 mL per 5 – 10 seconds) and into fresh collection tubes prepared in Step 1.
   Note: Pushing the EVs across the Minisart® too quickly may lead to lower recovery.

Treatment of Cells with Labeled EVs

1. Remove assay plate from incubator and perform a full media replacement with labeled EV containing media.
   1.1 Avoid leaving wells empty of media for extended periods of time as this can dry out wells and result in cell perturbation or death.
   1.2 It may be helpful to prepare the labeled EVs in fresh 96-well plate and use multi-channel pipettes for ease of transfer to the assay plate.
   1.3 Depending on uptake kinetics, it may be preferable to treat cells with compounds prior to adding EVs.
2. Remove any bubbles from wells (See General Guidelines) and place plate in the Incucyte® Live-Cell Analysis System.
   Note: Assay plate will undergo a full media replacement so scanning the plate prior to temperature acclimation of the media in the wells may cause condensation formation prior to first image acquisition. If immediate imaging is desired, place the plate in the Incucyte® Live-Cell Analysis System and perform a single plate scan to clear condensation. If immediate scanning is not necessary, allow the plate to acclimate for about 15 – 30 minutes prior to acquiring scans.

Acquire Images

1. Using the Incucyte® Software, schedule to collect "Phase" and "Green, Red or NIR" channel images (objective: 10X or 20X). Scanning frequency will depend on experimental conditions, but more frequent scanning (e.g. 30-minute intervals) may be desired during the first few hours of assay when cells begin to take up the labeled EVs.
2. Data acquisition using the Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031) is recommended for all applications. Data acquisition using Basic Analyzer may be possible for some applications but requires optimization of the assay.
3. To generate the metrics, user must create an Analysis Definition suited to the cell type, assay conditions and magnification selected.

Cell-by-Cell Analysis (Recommended)

1. Using Cell-by-Cell Analysis Software select Adherent Cell-by-Cell analysis type to create a new Analysis Definition.
   1.1 Select representative images from both treated and control wells. Ensure majority of images have ~50% cell coverage to enable accurate masking.
   1.2 Begin with optimizing the Cell Boundary (with fluorescent channels turned off) by determining the optimal Seed mask (goal is to have one seed per cell) using the slider controls.
   1.3 Evaluate the Cell-by-Cell Mask and refine the parameters accordingly. Once you are happy with the parameters, click "Preview All" to ensure that the analysis parameters are appropriate for other time points or treatments selected.
   1.4 Fluorescent signal is contained with the cell boundary so there is no need to generate a separate fluorescent mask. The default setting of Surface Fit No Mask for the fluorescence channel will enable background subtraction.
   1.5 Once you have previewed all the representative images and are satisfied with the parameters, complete the Launch Wizard analysis to select the Scan Times and Wells to be analyzed, as well as assigning an analysis definition name.
   Note: If your experiment is in progress, you will have an option to check "Analyze Future Scans" to perform real-time analysis.
2. Once cell segmentation has been completed, the data can be classified within the software based on green, red or NIR fluorescence intensity using a 2D scatter plot.
3. For further in-depth guidelines to aid with analysis, please see the User Manual within the Incucyte® software user interface.
4. As the labeled EVs are taken up by the cells, the fluorescence intensity inside the cells increases. See table below for recommended metrics.

Basic Analysis

1. To create a processing definition, select representative images from wells containing labeled EVs across 1 – 3 timepoints, and include a negative control well.
2. Set up the mask for the phase confluence measure with fluorescence channel turned off.
3. Once the phase mask is determined, turn the fluorescence channel on: remove background fluorescence from the mask using the background subtraction "Surface Fit" feature.
   3.1 The threshold chosen will ensure that objects below a fluorescence threshold will not be masked (it is recommended to select Edge Split Off for more complete masking).
   3.2 Choose a threshold in which green, red or NIR objects are masked in the positive response image but low in the dye only or negative control wells. Choose a threshold which results in masking the maximum cell area without extending beyond cell borders or picking up unwanted fluorescent background.
   3.3 The filter feature can be helpful to avoid masking cellular debris (e.g., selecting a minimum size filter).
4. As the labeled EVs are taken up by the cells, the fluorescence intensity inside the cells increases. See table below for recommended metrics.

Data Interpretation

	Cell-by-Cell Analysis	Basic Analyzer
Recommended Applications	▪ All applications ▪ Software segments cells in phase, using a processing definition across multiple experiments (containing the same cell type) is possible.	▪ Optimized conditions where background fluorescence is not limiting background subtraction. ▪ Processing definitions will need to be set-up across experiments as the fluorescent threshold may vary across treatments.
Increase in Green Average Mean Intensity	Green Average Mean Intensity (GCU). Post classification: Green Mean Intensity (GCU) | Object Count Per Well	Not recommended
An increase in fluorescence intensity, integrated over the area of detectable fluorescence	Not recommended	Green Total Integrated Intensity (per image) normalized to Phase Area (per image) (GCU x µm²/image ÷ µm²/image). Notes: Select "Edge Split OFF" for fluorescence masking. Use custom metrics to set analysis type.

Troubleshooting Guide

Weak or No Signal

- Uptake of labeled EVs may not be visualized in unprocessed images (or fluorescence may be faint). Applying a processing definition will remove background and enhance visualization of the signal.
- EV particles may not be in sufficient quantities to visualize uptake. A titration of labeled EVs can be tested to find a window that works for the desired end analysis.
  - To test "µg" of EVs, we recommend testing 1 – 4 µg per well
  - To test "particles per mL" (EVs with low protein concentrations), we recommend starting at a minimum of 50×10^6 particles per well and titrating upwards.
- EVs particles may have been sheared or disrupted during extraction process.
- Biology of exosome | uptake cell may prevent uptake of EVs.
- -eFBS may still contain a low concentration of exosomes. Among commercially available options, we recommend Gibco, Cat. No. A2720803. Using exosome-containing serum is strongly discouraged as it will affect the uptake of labeled exosomes.

High Background

- Loading more than 20 µL of Incucyte® Exofluor Dye (0.5 mM) on a single Vivaspin® 2 may lead to free dye carryover in the processed EVs. When using more than 20 µL of Incucyte® Exofluor Dye, it is highly recommended to run a "dye only" control to monitor for change in background GCU.
- An extra 2 mL PBS wash cycle of the Vivaspin® 2 may help bring the background down to minimize background signal from unincorporated dye.
- Additional purification of exosomes may be required if starting from a lower purity extraction method (e.g. PEG purification method).
- Some commercially available -eFBS sera has been found to introduce high background in the assay. Gibco exosome-depleted Fetal Bovine Serum (Cat. No. A2720801) has been most successfully used in this assay.

Improved Recovery for Low Volume (Yield)

- If recovery volume is less than 1 mL of media, it is recommended to first pre-wet the 0.2 µm filter with ~500 µL of media. Be sure to discard this media as thoroughly as possible before processing the EV sample. Use of a dry membrane results in a volume loss of at least 250 µL whereas pre-wetting will lead to a volume loss of 60 – 100 µL.

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Incucyte-Exofluor-EV-Kit-Product-Guide-en-Sartorius Status: 08 | 2025