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Real Time EV Uptake Characterization Using an Incucyte® Live Cell Imaging System

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Summary and Impact

- The Incucyte® Exofluor Green EV Labeling Kit provides a novel method for the real-time visualization and quantification of extracellular vesicle (EV) uptake in live cells.
- By employing a covalently bound dye, the kit offers stable and uniform labeling of EVs, representing a significant improvement over traditional labeling techniques and reducing background noise.
- The kit's compatibility with a variety of EV purification methods and cell origins ensures its broad applicability in live-cell assays, facilitating diverse research opportunities.
- The Exofluor Green Labeling Dye enables EV-specific visual tracking of EV uptake, allowing for precise studies of EV dynamics without the interference of non-specific dye attachment.
- Researchers can leverage the kit to investigate EV uptake and function, such as wound healing, to enable a deeper understanding of intercellular communication.
- Mesenchymal stem cell (MSC)-derived EVs, labeled with Exofluor Green, demonstrate uptake and kinetic migration effects using the Incucyte Scratch Wound Cell Migration assay,

Exofluor Green Labeling Method

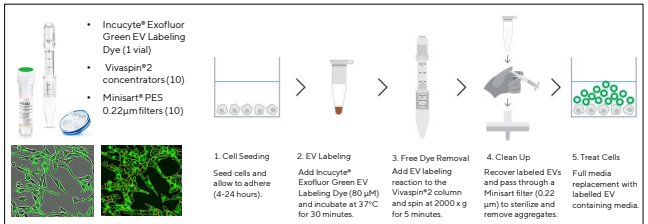

Figure 1: Quick Guide (Sartorius Kit BA-04877)

MSC-Derived EV Purification Process

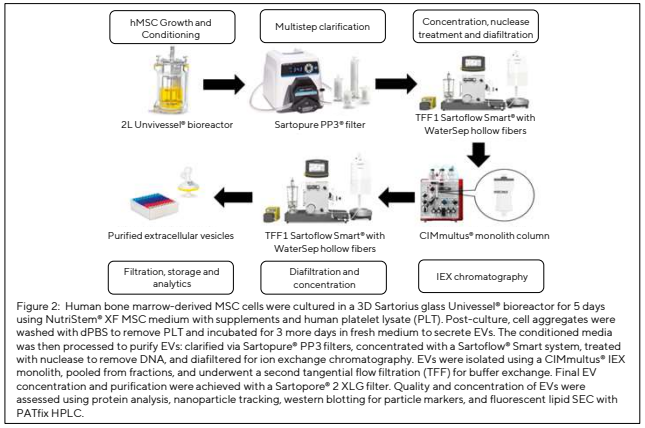

Figure 2: Human bone marrow-derived MSC cells were cultured in a 3D Sartorius glass Univessel® bioreactor for 5 days using NutriStem® XF MSC medium with supplements and human platelet lysate (PLT). Post-culture, cell aggregates were washed with dPBS to remove PLT and incubated for 3 more days in fresh medium to secrete EVs. The conditioned media was then processed to purify EVs: clarified via Sartopure® PP3 filters, concentrated with a Sartoflow® Smart system, treated with nuclease to remove DNA, and diafiltered for ion exchange chromatography. EVs were isolated using a CIMmultus® IEX monolith, pooled from fractions, and underwent a second tangential flow filtration (TFF) for buffer exchange. Final EV concentration and purification were achieved with a Sartopore® 2 XLG filter. Quality and concentration of EVs were assessed using protein analysis, nanoparticle tracking, western blotting for particle markers, and fluorescent lipid SEC with PATfix HPLC.

Validation of EV Labeling Kit

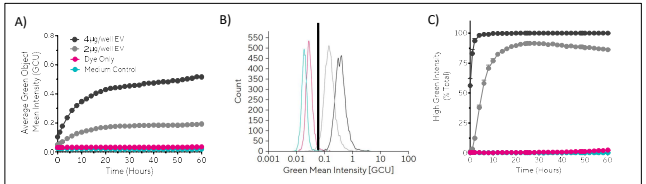

Figure 3: Data Analysis Tools to Measure Uptake of Exoflour Green Labeled EVs. (A) The Average Green Object Mean Intensity increased as a measure of the A549 cells taking up labeled, PC-3 exosomes. (B) Histogram analysis is used to classify populations of segmented cells at 24-hours. (C) Quantification of the percentage of labeled cells, classified as having high green intensity, over time (mean ± SEM, n=4).

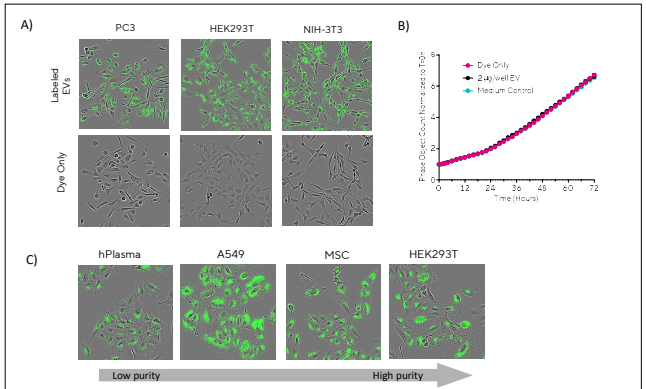

Figure 4: Uptake Cell and EV Labeling Compatibility. (A,top) A549 HansaBioMed Exosomes were labeled using the Incucyte® Exofluor Dye and cell lines were treated with labeled exosomes. (A, bottom) Dye only control wells for each condition demonstrate the fluorescent signal is not due to residual free dye after processing. Phase images show no change in cell morphology compared with controls. Images shown taken at 24-hours post-treatment. (B) Object Count normalized to T=0h (mean ± SEM, n=4) illustrates no change in proliferation of PC-3 cells across all conditions. (C) Images demonstrate uptake of various EVs labeled with Incucyte® Exofluor Green by A549 cells (24 hours post-treatment). Human plasma-derived exosomes (Abcam, ultrafiltration) and A549 exosomes (ultrafiltration and SEC) were labeled and added to cells at 4 and 2 µg/well, respectively. Both MSC and HEK293T EVs were extracted using TFF /ion exchange chromatography (in-house) and treated at 1.5 E8 and 8.3 E7 particles/well, respectively.

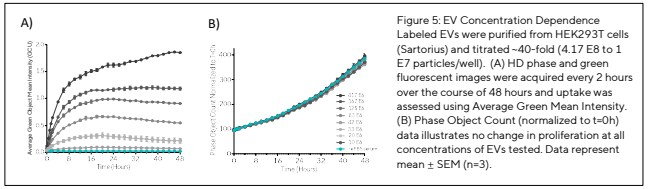

Figure 5: EV Concentration Dependence Labeled EVs were purified from HEK293T cells (Sartorius) and titrated ~40-fold (4.17 E8 to 1 E7 particles/well). (A) HD phase and green fluorescent images were acquired every 2 hours over the course of 48 hours and uptake was assessed using Average Green Mean Intensity. (B) Phase Object Count (normalized to t=0h) data illustrates no change in proliferation at all concentrations of EVs tested. Data represent mean ± SEM (n=3).

Mechanism of Action

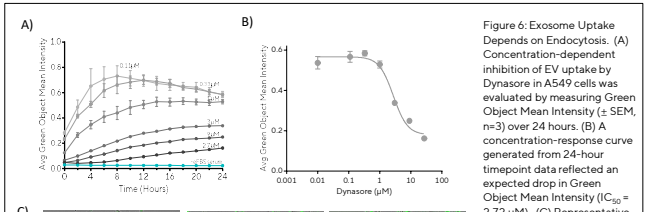

Figure 6: Exosome Uptake Depends on Endocytosis. (A) Concentration-dependent inhibition of EV uptake by Dynasore in A549 cells was evaluated by measuring Green Object Mean Intensity (± SEM, n=3) over 24 hours. (B) A concentration-response curve generated from 24-hour timepoint data reflected an expected drop in Green Object Mean Intensity (IC_{50} = 2.72 µM). (C) Representative images (24-hour timepoint) provide visualization of Dynasore inhibition of EV uptake at the indicated concentrations.

Scratch Wound Migration

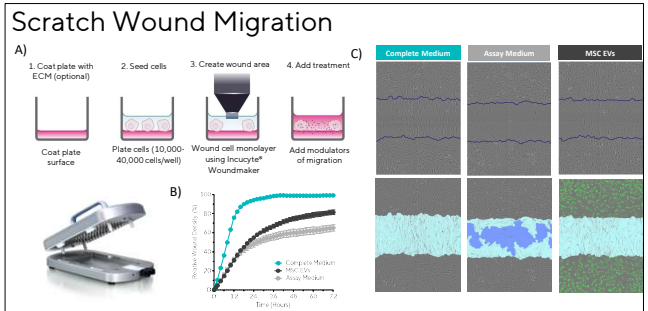

Figure 7: Scratch Wound Migration using Incucyte® Live-Cell Imaging. The Incucyte® Woundmaker Tool was used according to standard protocols and the Incucyte® Scratch Would Analysis Software Module enabled visualization and quantification of migration effects. NHDF Cells were seeded (15K cells/well) on collagen-coated plates, scratched, and media was replaced with Complete Medium (DMEM + 10% FBS), Assay Medium (serum free DMEM) or Assay Medium containing MSC-derived EVs (4.5 E9 particles/well). (A) HD Phase images showed varying degrees of cell migration into the initial wound at 18-hours post scratch. *Top row*, Phase only images with initial wound boundary shown in blue. *Bottom row*, Phase + Green fluorescence imaging shows MSC-derived EV uptake and Wound Density changes (masking highlights RWD). (B) Cell migration was captured using an Incucyte® Live-Cell Analysis System and relative wound density (mean± SEM, n=6) was quantified over time.

Conclusions

- The Incucyte® Exofluor Green EV Labeling Kit enables real-time visualization and quantification of EV uptake in live cells with a stable, covalently bound dye.
- Validated across various EV purification methods and cell types, the kit facilitates the study of EV uptake mechanisms and their functional roles, such as in wound healing.
- The kit's specificity allows for accurate EV tracking in live-cell assays, supporting standardized research into EV dynamics and functions.