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EV Isolation at Exceptional Levels of Purity and Yield

ExoQuick® ULTRA & ExoQuick-TC® ULTRA

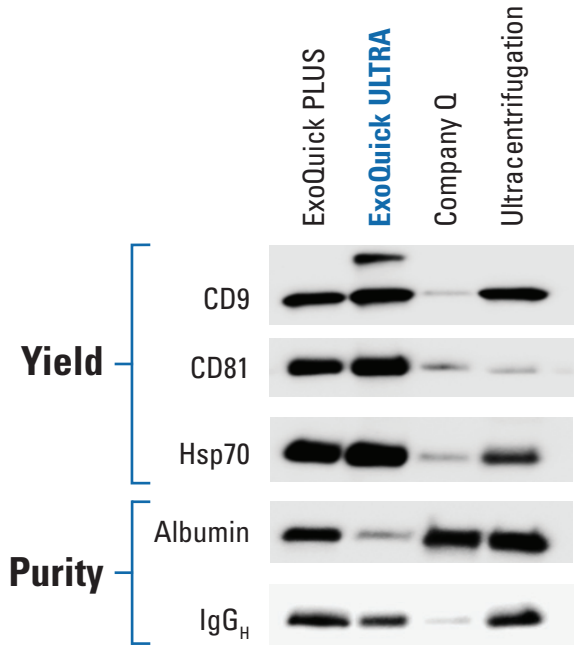
ExoQuick® gets even better

Drawing upon years of exosome experience, the SBI team has pushed ExoQuick extracellular vesicle (EV) isolation technology to new peaks of performance with ExoQuick® ULTRA for Serum & Plasma and ExoQuick-TC® ULTRA for Tissue Culture Media and other biofluids. While many EV isolation methods require you to choose between high yields, easy protocols, clean preps, and low costs, ExoQuick ULTRA and ExoQuick-TC ULTRA are able to deliver on all these fronts for trade-off free EV preparation.

From as little as 250 µL of serum or plasma or 5 mL of tissue culture media or other biofluid, you can get high quality EVs for a wide range of downstream applications such as such as western blotting, mass spectrometry, NGS sequencing, exosome labeling, and *in vivo/ex vivo* exosome delivery.

ExoQuick ULTRA delivers high yields of clean exosomes

Comparison of different exosome isolation methods demonstrates the high yields and lower levels of carry-over proteins possible when using ExoQuick ULTRA compared to ExoQuick PLUS, a competitor's column-based kit, and even ultracentrifugation.



The ExoQuick ULTRA prep contains the highest levels of the exosome-specific markers CD9, CD81, and Hsp70 and the lowest levels of the carryover proteins albumin and IgGH.

In contrast, the prep from Company Q appears to be primarily albumin, and even the sample prepared using ultracentrifugation contains considerably higher levels of both albumin and IgGH.

METHODS: Each lane was loaded with 7 µg of total protein as measured using a fluorometric Qubit protein assay.

Learn more about ExoQuick ULTRA and ExoQuick-TC ULTRA by visiting ***systembio.com/ExoQuick-ULTRA***

Real Talk: Exosomes as the Cell's CCO

"They are an essential form of cellular communication that was hidden in plain (but microscope-aided) sight"

As any bibliophile can tell you, every good protagonist has struggled to overcome long odds to slay a dragon, whether real or metaphorical. The same is true of exosomes. Early understanding of exosome function was that of a cellular waste disposal pathway, trash packaged as lipid-based vesicles to prevent it from sparking an immune reaction. For decades, exosomes were purified away from the other, more valuable cellular components, until it was realized that exosomes don't just carry a random assortment of cellular refuse; they carry messages from cells to other cells.[1] They are an essential form of cellular communication that was hidden in plain (but microscope-aided) sight, and scientists are now learning how to interpret and influence the messages they contain for diagnostic and therapeutic purposes.

Exosomes Everywhere

With few, if any, exceptions, exosome-based communication is exhibited by all mammalian cell types that have been studied. While there are variations in the exosome membrane components and their contents, or cargo, exosomes are typically 50-150 nm in diameter. Other larger extracellular vesicles (EVs) exist, but research suggests that they are distinct from exosomes not only in size, but also in biogenesis and function.[2] From breast milk to blood, exosomes show up in a whole host of bodily fluids and participate in intercellular communication processes.[3] It's not surprising that exosomes are an attractive focus of research in many laboratories.

Direct Address

The extracellular milieu is full of exosomes released by cells, some signaling health, some signaling distress, and others just letting their neighbors know that they're still there. Exosomes released locally can be found in high concentrations in the cytosol immediately surrounding a target cell, but exosomes from far-flung cells in distant organs can travel through the circulation to fluid-filled spaces throughout the body.[4] Liquid biopsies are a method for non-invasively sampling exosomes from bodily fluids including urine,[5] cerebrospinal fluid,[6] saliva,[7] synovial fluid,[8] and tears,[9] to name just a few.

Curious About Cargo?

The composition of each exosome, from membrane to cargo, is dependent on its origin, biogenesis, and function. Exosomes are involved in various biological functions including the regulation of the immune system,[10] tumorigenesis,[11] and the nervous system,[12] and their cargo is heterogeneous to boot; they carry DNA, protein, lipids, mRNA and microRNA. It's no wonder that, at first glance, the proposed function of exosomes seems endless. The interaction of multiple cargo components is especially curious in exosomes since subtle variations in content often leads to perturbations in homeostasis. Indeed, the study of exosome contents is the subject of intense research and plenty of industrial and academic investigations are looking into delineating the mechanisms regulating exosome cargo and membrane production.

Future Forecast

As techniques for isolating exosomes from human samples such as liquid biopsies improve, it's reasonable to expect that methods for characterization and analysis will improve in tandem. Exosomes have tremendous potential for diagnostics and potentially for novel therapeutic strategies. Biopharmaceutical endeavors looking to harness the natural delivery capabilities of exosomes can develop novel platforms for targeted drug delivery for a plethora of diseases. Multidisciplinary approaches are most likely to make the biggest gains when it comes to developing exosome platforms, which address some of the most critical challenges in health care. The future is certainly exciting for basic exosome research insights as well as biomedical commercialization ventures.

For references, please see page 6.

Context Clues: Research Applications of Exosomes

"There is a substantial gap in the therapeutic delivery world when it comes to delivering genetic materials in a safe and targeted way."

Beyond their role as the best kept cell-signaling secret of the last century, it stands to reason that these microscopic messages also contain details about disease states, giving clues that signal disease onset, progression, and remission. On top of that, their lipid membranes are exquisitely suited to permit stealthy but targeted therapeutic delivery. As such, exosomes are now taking their place at the center of disease research and therapeutic development.

Diagnosis & Prognosis

As powerful mediators of paracrine signaling, exosomes carry all the machinery necessary to change the behavior of neighboring cells. There is plenty of *in vitro* evidence to demonstrate that exosomes are released by specific cells in a manner that reflects their function. This includes synaptic activity in neuronal cells,[1] hypoxia in endothelial cells,[2] and cell-cell contact in fibroblasts.[3] This has led to several clinical investigations seeking to translate exosome involvement in cell communications into important biomarkers when evaluating the diagnosis and prognosis of various pathologies. There are already reports of exosomes being used in breast,[4] prostate,[5] and non-small cell lung[6] cancer patients. Circulating exosomes are being used as less invasive type of biopsy to predict factors such as distant organ metastasis, chemotherapy resistance, tumor reoccurrence, and overall survival.

Discrete Delivery

If exosomes make such great couriers, carrying their goods from cell to cell, it stands to reason that they can also deliver freight that is not so welcome. Among other diseases, they have been shown to be implicated in the exchange of miRNAs in cancer,[7] amyloid-β in Alzheimer's disease,[8] prions in spongiform encephalopathies,[9] and tau in neurodegenerative disease.[10] However, their ability to deliver such diverse packages also makes them an exemplary platform for delivering therapeutics.

Currently used drug delivery systems include liposomes and polymeric liposomes. These systems have been used to deliver various therapeutics including anti-cancer drugs, anti-fungal drugs, and painkillers.[11] However, their relative inability to evade the immune system in the case of liposomes, and their biocompatibility and long-term safety in the case of polymeric nanoparticles leaves a niche in the drug-delivery market for exosomes to potentially fill.

Pocket-sized: Small Molecules

Small molecules are perfectly suited to be gift-wrapped up and sent out for special delivery by exosomes. Research has so far shown a number of small molecules can be transported via exosomes, including: Curcumin, a natural polyphenol found in turmeric with anti-inflammatory and chemo-preventative properties, to treat inflammatory disease;[12] anti-cancer drug doxorubicin into tumor tissues;[13] and doxorubicin along with fellow anti-cancer drug paclitaxel across the blood-brain barrier.[14]

The Big Guns: Proteins

Able to dip their fingers into many pies, exosomes are not only capable of delivering small molecules, but also large molecules such as proteins and peptides. In another blood-brain barrier busting feat, exosomes were recently shown to successfully deliver the antioxidant protein catalase – a superhero in the world of free radical deactivation – to the brains of patients suffering from Parkinson's disease.[15]

Genetic Bullets: Nucleic Acids

There is a substantial gap in the therapeutic delivery world when it comes to delivering genetic materials in a safe and targeted way. Exosomes' unique ability to deliver nucleic acids from cell to cell through fluid to fluid, while also avoiding the host's immune system, makes them a natural target for research in gene therapy. However, this area still requires much more research. Preliminary research has shown that small interference RNA (siRNA) can be delivered to the brains of mice,[16] as well as to T cells and monocytes.[17] And exosomes have also been used to deliver anti-tumor miRNA (short non-coding RNA) to breast cancer cells.[18] Hopefully, it's only a matter of time until exosomes show their full potential in this area of research.

For references, please see page 6.

WORK(FLOW) IT OUT

A MINI WORKFLOW FOR MINI MESSENGERS

COLLECT

Liquid biopsy is a popular way to collect these ubiquitous bundles from bodily fluids such as serum, plasma, urine, CSF, saliva, etc.

ISOLATE

From polymer precipitation to size exclusion chromatography to ultracentrifugation, there are multiple ways to purify these miniature messengers

CHARACTERIZE

Measure the size, concentration and contents of exosomes with NTA, Western blots, TEM, etc.

STUDY FUNCTIONALITY

Visualize, load with cargo, and track to determine impact of exosomes

ENGINEER/DELIVER

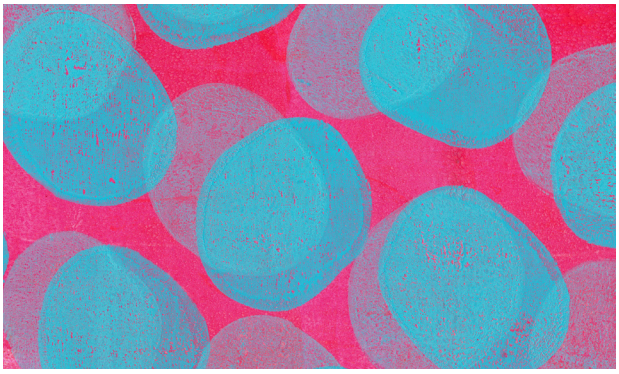
Load with proteins or other cargo and target specific areas/organs

DISCOVER BIOMARKERS

Using approaches such as Mass Spec or NGS, researchers can obtain clues on disease onset, progression and remission, and can also use exosomes to diagnose

So, You Want to Study Exosomes? Key Parameters and Considerations

"As new exosome isolation techniques become more cost-effective and readily available, research into exosome biology will continue to grow."



Now that you have a fuller appreciation for what these little bundles of macromolecular messages are and what they can do for you, it only seems fair to equip you with the key considerations for isolating and characterizing them. The following tips and parameters will help you to plan your first, or next, foray into the world of exosomes.

Isolation

There are several popular techniques for isolating exosomes, and one's choice will depend heavily on the exosomes' downstream use and source sample volume. For highly pure, intact exosomes, differential ultracentrifugation is considered the gold standard method,[1] however it may miss as many as 50% of the total exosomes in the sample; this math may be acceptable if the starting volume is high. For smaller volume samples, both precipitation and size-exclusion chromatography are gaining popularity,[2] but they do not distinguish between particles in the exosome size range. Antibody-based methods require a physical association with the exosome, something that may alter the exosome's biophysiology, as well as prior knowledge of the exosome's membrane composition. There are also concerns about exosome damage and cargo loss upon releasing an antibody-bound exosome from the isolation substrate (e.g., beads, magnetic beads).[3] Do your homework before choosing the right method for your lab.

Characterization

Exosome membranes appear to retain a stereotypical composition between cell types, and it is this composition that is the basis for exosome characterization. By probing exosome samples, either intact or homogenized, the presence of key membrane proteins can be used as an internal standard. Exosome membrane analysis typically involves electron microscopy, immunoflurorescence, flow cytometry, or Western blotting.[4] Isolated exosomes often stick to one another and show up as distinct aggregates using conventional flow cytometry or dynamic light scattering.[5] Commonly, the tetraspanin transmembrane family of proteins, which includes CD63, CD9, and CD81, are commonly utilized markers used to identify exosome vesicles. Measuring the expression of just one of these proteins may not be sufficient since the levels of individual tetraspanins vary greatly between different types of exosomes.

The International Society for Extracellular Vesicles recommends measuring at least one tetraspanin marker in combination with endosomal membrane binding proteins such as Alix or TSG101.[7] Furthermore, the lack of classic intracellular proteins such as HSP90B1 may also used to demonstrate that isolated samples contain characteristic exosomes that are free from other contaminants. Cargo characterization, which requires sample homogenization, depends largely on the targets of interest, as sample prep will differ if you're searching for nucleic acid targets, protein targets, or lipid targets.[8]

Processing

Isolation of highly pure exosome fractions can be used for several *in vitro* and *in vivo* experiments. However, isolated exosomes should be stored carefully under sterile conditions to keep the exosomes viable. Structural integrity of the vesicle surface is of utmost importance when it comes to determining functional efficacy.[9] This is especially true if you are preparing exosomes for electroporation or subjecting them to post-isolation modifications including nanoparticle conjugations.[10] For ideal results, it is best to use freshly isolated exosomes for subsequent experiments and analysis. Despite exosomes being remarkably stable, their protein content and cargo functionality is still subject to degradation when they are stored in either 4°C or -80°C conditions.[11] When fresh isolations are not possible, a good practice is to supplement the storage media with a cocktail of protease inhibitors to prevent surface protein degradation.

As new exosome isolation techniques become more cost-effective and readily available, research into exosome biology will continue to grow. There is going to be an increase in its applications for clinical diagnostics in the form of biomarkers, alongside therapeutic platforms when delivering exosome cargo including nucleic acids and proteins.

For references, please see page 6.

Article 1 - Real Talk: Exosomes as the Cell's CCO

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Article 2 - Context Clues: Research Applications of Exosomes

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Article 3 - So, You Want to Study Exosomes? Key Parameters and Considerations

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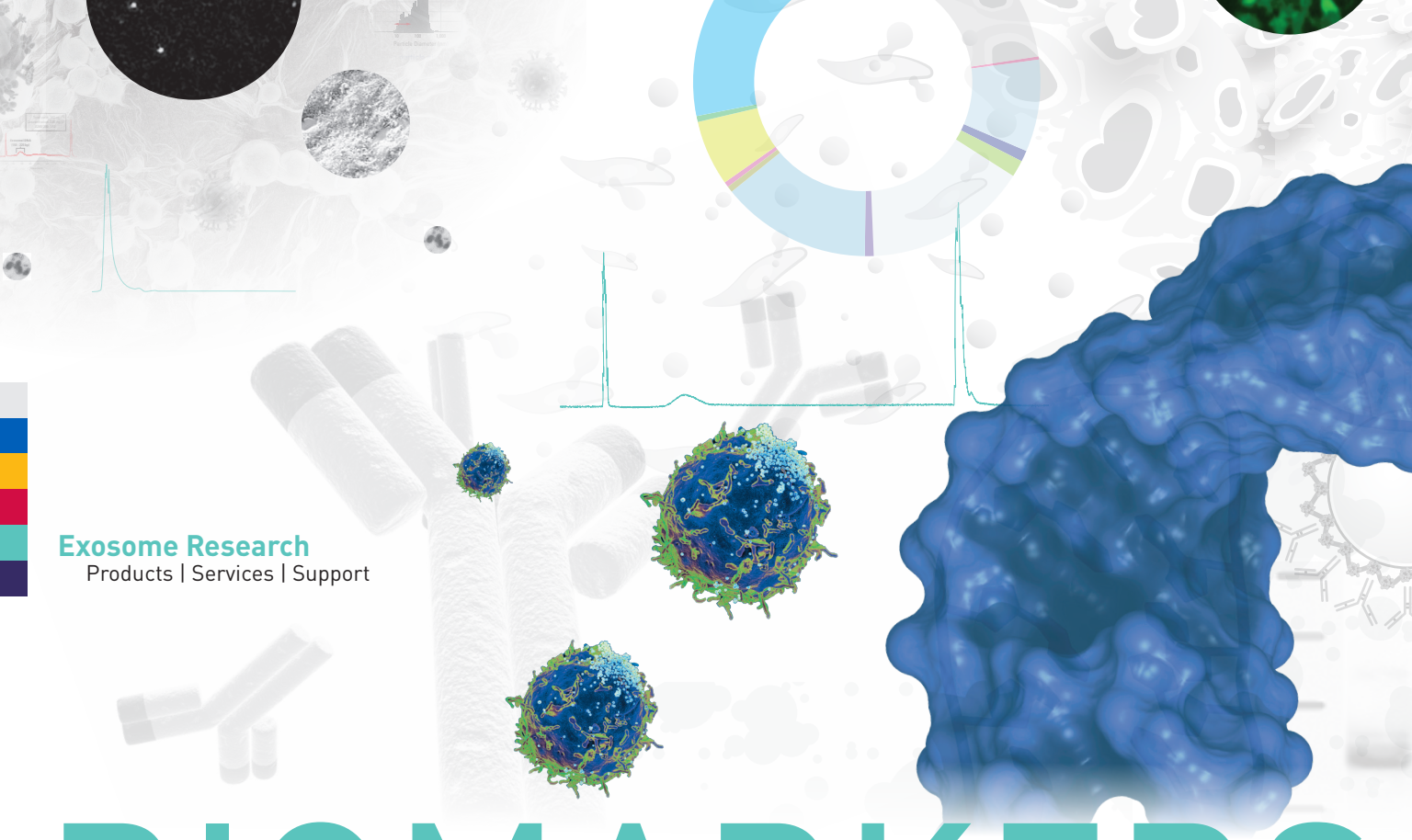

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