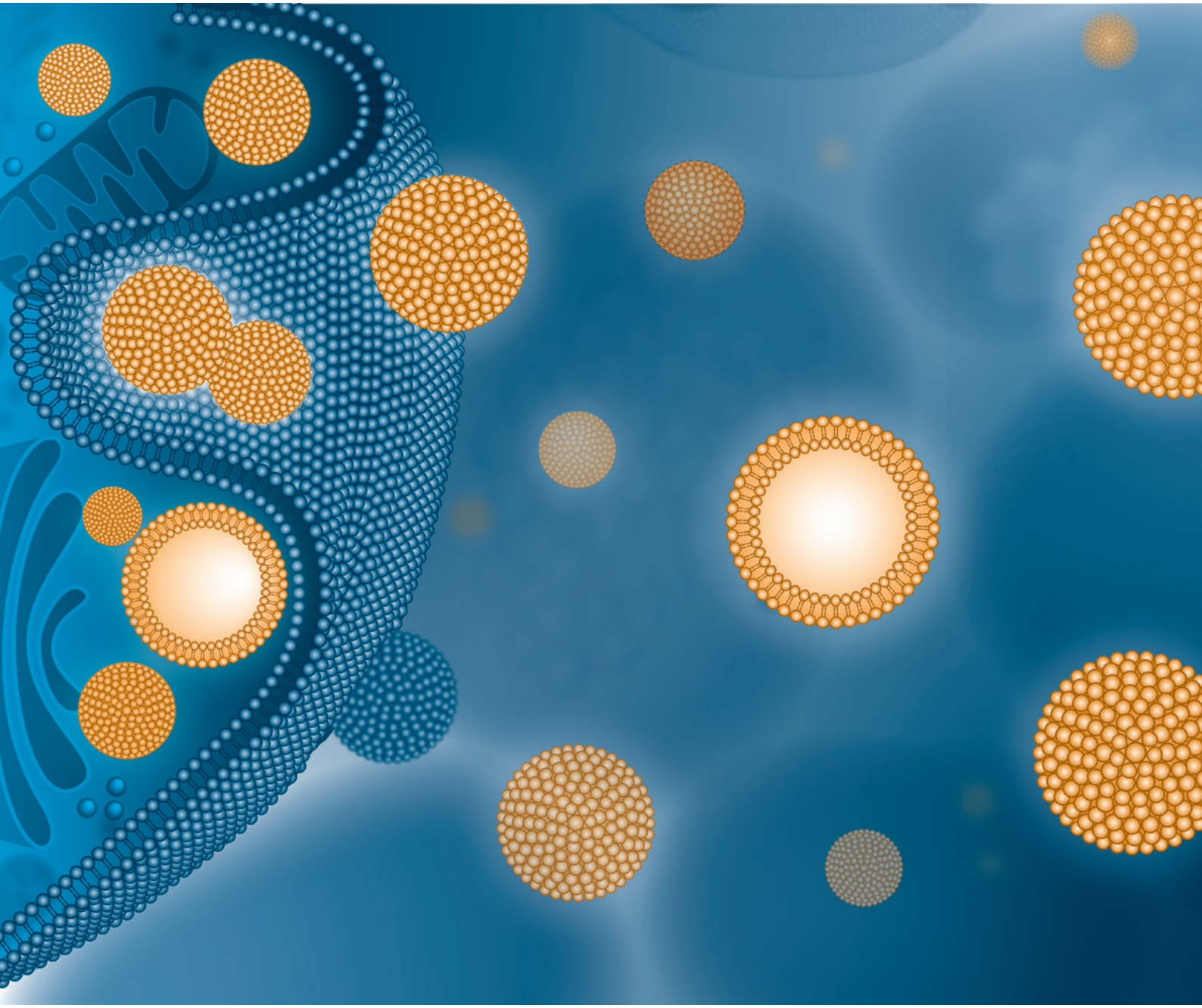
iba Solutions For Life Sciences

Cell Selection & Expansion

FAB-TACS® EXOSOME ISOLATION

Traceless Affinity Selection



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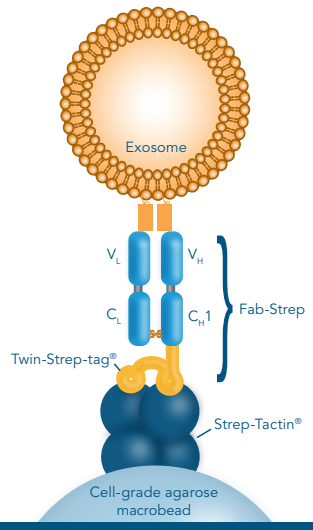
Exosome key facts

- Range between 30 - 150 nm
- Smaller than apotopic bodies and microparticles
- Surface contains tetraspanins CD9, CD81 and CD63
- Mediate cell-to-cell communication
- Therapeutic agents in multiple disease models

Key features

- High purity of exosomes up to 99%
- Quick and simple protocol
- Isolation in source independent manner
- Free of antibodies and magnetic beads
- Highly defined isolation with minimal contamination
- Capacity 5x10^{10} particles/ml bedvolume
- Reversible capture reagent



Fab-TACS® technology: non-magnetic, Fab-based immune affinity chromatography

EXTRACELLULAR VESICLES

Exosomes have an endosomal origin and are released by many different cell types, participating in different physiological and/or pathological processes. They range between 30 and 150 nanometers in diameter and belong to the smallest extracellular vesicles. Exosomes possess surface proteins that partly originate from plasma membranes during endocytosis. The tetraspanins CD9, CD63 and CD81 are specially enriched in the membrane of these vesicles and are often used as biomarkers. Exosomes transfer specific biomolecules such as DNA, RNA, proteins, enzymes and lipids and thereby mediate cell-to-cell communication. This intercellular vesicle traffic allows rapid and controlled communication playing an important role in many aspects of human health and disease, including development, immunity, tissue homeostasis, cancer, and neurodegenerative diseases.

Due to the small size of exosomes that partly overlaps with other extracellular vesicles such as apoptotic bodies (100 – 5000 nm) or microparticles (100 - 1000 nm), their isolation remains challenging. Currently used purification methods include differential centrifugation, size exclusion chromatography, filtration or polymer based precipitation (e.g. polyethylene glycol (PEG)ylation). Varying efficiencies depending on exosome source, contamination with other particles, low recovery of exosomes and time consuming isolation steps often limit the application of those methods. The development of surface marker specific magnetic bead isolation already improves the purification process, but impairs exosome integrity due to non-reversible binding to magnetic beads. In contrast, the Fab-TACS® exosome isolation technology allows the quick isolation of pure and unlabeled exosomes in a source independent manner.

TRACELESS AFFINITY SELECTION

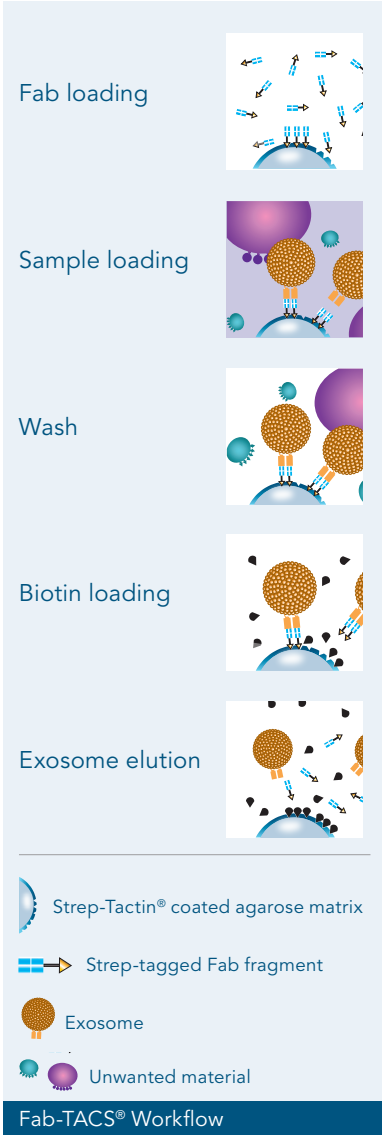
The Fab-based traceless affinity cell selection (Fab-TACS®) technology represents an affinity chromatography system for non-magnetic isolation of exosomes. It is based on Twin-Strep-tagged CD-specific Fab fragments (Fabs), which reversibly capture and release the exosomes. The technology delivers label-free exosomes with intact biological functions in a standardized manner with highly reproducible quality. The exosomes can be isolated from different cell culture supernatants, serum and plasma.

SIMPLE WORKFLOW

Columns for exosomes are filled with a Strep-Tactin® coated matrix made of cell-grade agarose. Strep-tagged low affinity Fab fragments (Fab-Streps) specifically bind to the matrix. Subsequently, cell culture supernatants, serum, plasma or other preparations pass through the column. Exosomes adhere to the matrix based on the exclusive binding of the Fab-Strep to the target. Unwanted material is efficiently washed away. In a final step, the addition of biotin causes the elution

of the exosomes and the Fab-Streps due to the higher affinity of biotin to Strep-Tactin®. After elution, the Fab-Streps self-dissociate from the vesicle surface. The label-free authentic exosomes are now ready for further downstream applications.

In case ultra-pure exosome preparations without Biotin and Fabs are required, size exclusion chromatography or hydrostatic filtration dialysis can be performed.



Fab-TACS® Workflow

HIGH QUALITY EXOSOME ISOLATION

Minimal contamination by other EVs

The particle size is a critical factor in evaluating the quality of isolated exosomes. 99% of particles isolated from cell culture supernatants of mesenchymal stem cells (MSCs) fell within the range of 30 - 150 nm (**A**). This indicates strong exosome enrichment. In comparison, only 32% exosome-sized particles were detected after purification with a commercially available PEGylation kit (**B**), implying contamination by larger extracellular vesicles.

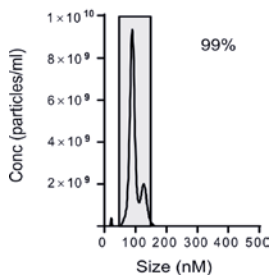
High content of exosome proteins

Since other same-sized non-exosome contaminants may contribute to the pool of isolated particles, the vesicles purified by our Fab-TACS® isolation technology from MSC supernatants were tested for the presence of marker proteins CD63 and Alix. Both proteins were clearly present within the purified particles (**C**), confirming their exosome phenotype.

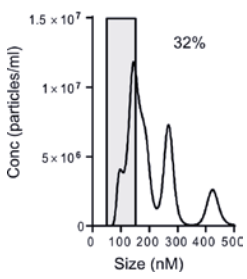
Reproducible results

Depending on MSC donor, cell culture supernatant compositions may vary. Purified exosomes of three independent isolations from different MSC donors were very comparable in their size ranging from 86 nm to 90 nm average diameter. All isolations yielded around 94% of particles between 30 and 150 nm in size (**D**). Besides high purity, this demonstrates a high reproducibility of our Fab-TACS® exosome isolation technology.

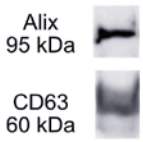
(A) CD81 Fab-TACS®



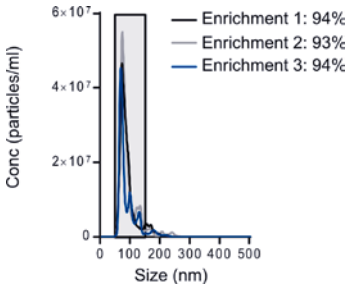
(B) PEGylation



(C) CD81 Fab-TACS®

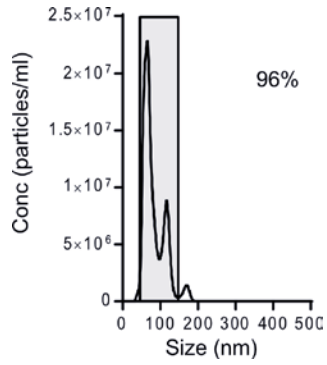


(D) CD9 Fab-TACS®



(A) Exosomes of MSC supernatant were isolated with CD81 Fab-TACS® exosome isolation kit. **(B)** Exosomes of MSC supernatant were isolated using PEGylation. **(C)** Exosomes were isolated using the CD81 Fab-TACS® kit. Western Blot was performed to analyze the protein content. Alix and CD63 specific mAbs were used to detect their target antigen. **(D)** Exosomes of three different MSC supernatants were isolated with CD9 Fab-TACS® exosome isolation kit. Data were analyzed with NanoSight LM10 and processed using NTA software 2.3.

(E) CD81 Fab-TACS®



(E) Exosomes were isolated from human serum using the Fab-TACS® technology targeting tetraspanin CD81.

Source-independent application

Important exosomes sources also include a variety of human bodily fluids such as blood and urine. Similar to isolations from cell culture supernatants, 96% of particles purified from human serum exhibited the typical exosome size of 30 to 150 nm (**E**).

Quick and simple

Due to the surface protein specificity of the Fab-TACS® isolation technology, time-consuming centrifugation steps or extensive sample preparations are not necessary. The protocol is easy and straightforward, permitting efficient and high quality exosome isolation also for newcomers in the field of exosome research. Additionally, the short processing time minimizes the stress of cargo contained within the exosomes.

Fab-TACS® exosome isolation technology delivers pure and specific exosome populations with highly reproducible results applicable for a broad range of exosome sources.

AVAILABLE ISOLATION KITS

Fab	Product description
CD9 human	CD9 Fab-TACS® Exosome Isolation Kit
CD81 human	CD81 Fab-TACS® Exosome Isolation Kit
CD9 human	CD9 Fab-TACS® Exosome Isolation Introductory Kit
CD81 human	CD81 Fab-TACS® Exosome Isolation Introductory Kit
CD9 / CD81 human	CD9/CD81 Fab-TACS® Exosome Isolation Starter Kit

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