iba Solutions For Life Sciences

Cell Selection & Expansion

Application Note

ISOLATION OF EXOSOMES FROM JURKAT CELLS AND HUMAN SERUM

High quality exosome isolation with Fab-TACS® technology

Introduction

The communication between different cells is essential for maintaining tissue homeostasis. When tissue injury occurs, an efficient interaction of immune cells with damaged tissue is required to resolve the inflammation and initiate wound healing. This interaction is either direct via cell-to-cell contact due to binding of ligands to receptors or indirect via secreted factors such as cytokines. In recent years, the release of exosomes from various cells was discovered as a novel cell-to-cell communication pathway.

Exosomes are endosome-derived small (30-150 nm) extracellular vesicles that contain various molecules such as nucleic acids or lipids. After their release, they carry information to neighboring or distant cells. In addition to their small size, exosomes can be characterized by surface proteins such as the tetraspanins CD81, CD9, or CD63[1–3].

Exosomes were identified as promising clinical treatment option due to their immunomodulatory and anti-inflammatory properties. They have been linked to eliciting innate and adaptive immune reactions. This supports their utility for therapy development and their potential role in coordinating immune reactions in response to infectious agents or cancer[4]. For example, seminal plasma-derived exosomes inhibit HIV-1 infection[5] and those produced by human dendritic cells promote a T helper 1 response[6]. Further, exosomes enhance antibacterial immune reactions by promoting bacterial antigen presentation[4]. Due to the multitude of cellular origin and cargo, exosomes have been implicated in almost all steps of the immune response.

To understand the role of exosomes in the communication within an immune response, it is crucial to isolate pure and functional exosome populations. Our Fab-TACS® technology for surface protein-specific exosome isolation allows the quick and efficient extraction of these vesicles from different samples. In this application note we will demonstrate that our Fab-TACS® isolation technology for $CD9^+$ and $CD81^+$ exosomes is applicable for cell culture supernatant of Jurkat cells and human serum, both critical systems to understand ongoing immune responses.

Materials and Methods

Human serum was obtained after 2 hours coagulation of whole blood and subsequent centrifugation at 3000 x g for 10 minutes twice. Serum and supernatants were filtered through a 0.2 µm polyethersulfone filter before the isolation procedure. Exosomes were isolated according to the CD9 and CD81 Fab-TACS® exosome isolation manuals.

Jurkat cells were cultured in RPMI 1640 + 2 mM Glutamine + 10% Fetal Bovine Serum (FBS) medium at 37°C with 5% CO_2. At 80-90% confluence in T75 flasks, cells were washed and serum-free medium (Starvation condition, < 2% exosome-free FBS, produced by 24 h centrifugation at 110.000 x g) added. After 24 h, supernatant was collected and centrifuged for 10 min at 3000 x g. As comparative methods, exosomes were isolated from Jurkat cell culture supernatants using size exclusion chromatography (according to supplier's manual), filtration (according to supplier's manual), and ultracentrifugation.

Isolated particles were analyzed with the NanoSight LM10 instrument (Malvern Instruments), and data were processed using NTA software 2.3. Protein content was determined by Western Blot analysis after

Jurkat cells

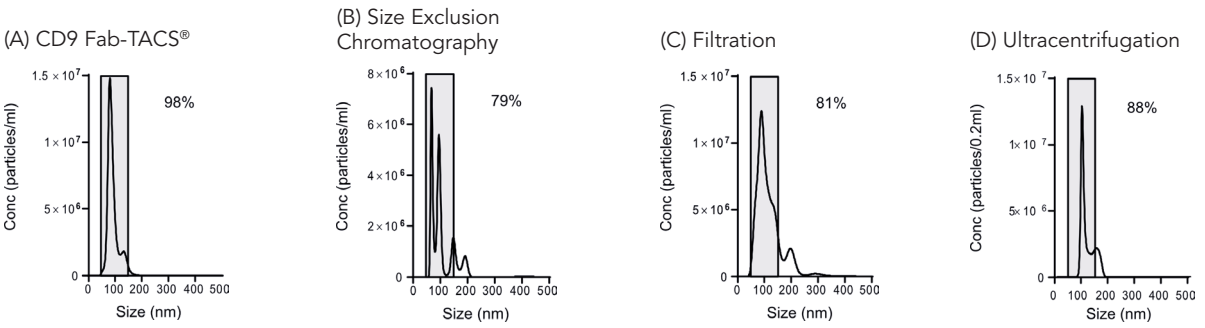

Fig. 1: (A-D) Exosomes were isolated from Jurkat cells using the CD9 Fab-TACS® exosome isolation technology (A), size exclusion chromatography (B), filtration (C) or ultracentrifugation (D). The extracellular vesicles isolated were analyzed with the NanoSight LM10 instrument (Malvern Instruments) and data were processed using NTA software 2.3 (A,B). The gray windows show the target size for exosomes.

thermo-lyses of isolated exosomes.

Results and Discussion

MSCs were incubated at 37°C in a humidified atmosphere with 5% CO_2. Adherent cells were allowed to expand until they reached about 80% confluence. Morphology of isolated and expanded MSC cells was assessed during the cell culture period using light microscopy and FACS analysis.

For exosome production and harvest cells were cultivated in starvation medium up to 72 h (starvation condition, < 2% exosome-free human platelet lysate (hPL)). After the starvation period cell supernatant was collected and exosomes isolated using a Fab-TACS® Exosome Isolation Kit.

Jurkat cells

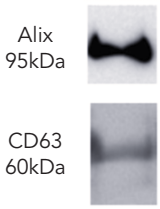

Fig. 2: Exosomes were isolated from Jurkat cell culture supernatant using the Fab-TACS® technology targeting tetraspanin CD81 on the surface of exosomes. Exosomal proteins Alix and CD63 were analyzed using Western Blot.

To further confirm the exosome phenotype of particles enriched with the Fab-TACS® exosome isolation technology, we tested for the presence of marker proteins Alix and CD63. Both proteins were detectable within the particle population (Figure 2), confirming their exosome nature.

Extracellular vesicles are also found in a variety of different bodily fluids, such as blood and urine. Exosome-specific purifications from those samples prove to be even more challenging due to interfering small-sized particles such as very low-density lipoprotein (VLDL). We used our CD9 and CD81 Fab-TACS® Exosome Isolation Kits to extract exosomes from human serum. Targeting tetraspanin CD81 resulted in the enrichment of particles, of which 96% were exosome-sized (Figure 3A) and slightly lower if CD9 specific Fabs were used (Figure 3B). Particles isolated with

Human serum

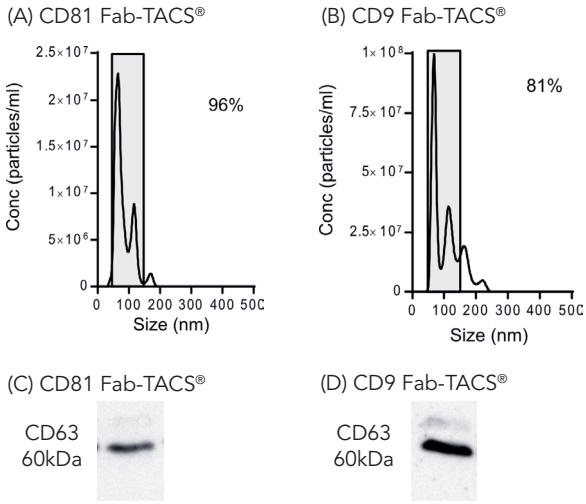

Fig. 3: (A-D) Exosomes were isolated from human serum using the Fab-TACS® technology targeting the tetraspanins CD81 (A,C) and CD9 (B,D) on the surface of exosomes. The extracellular vesicles isolated were analyzed with the NanoSight LM10 instrument (Malvern Instruments) and data were processed using NTA software 2.3 (A,B). The gray windows show the target size for exosomes. Exosomal proteins Alix and CD63 were analyzed after CD81 (C) or CD9 (D) Fab-TACS® exosome isolation using Western Blot.

both kits contained exosome marker protein CD63 (Figure 3 C, D). This shows that our Fab-TACS® technology is also applicable for more complex samples such as human serum.

Conclusion

Our CD81 and CD9 Fab-TACS® exosome isolation technology is suitable for a broad range of different samples that vary in their complexity. It guarantees the extraction of label-free, functional exosomes that can be used for various downstream applications.

References

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