ExoMAX Opti Enhancer™

Cat# EXOMAX12A-1

Cat# EXOMAX24A-1

User Manual

Store at +4^{0}C or +25^{0}C

Do not freeze

Version 1

1/24/2017

Contents

Product Description

ExoMAX Opti Enhancer™ is a novel formulation designed to enhance the existing workflow for OptiPrep™ gradient users to obtain highly pure exosomal preps. By replacing the initial differential ultracentrifugation step in the traditional OptiPrep gradient protocol with a simple, overnight incubation, a major pain point in the workflow is relieved (Fig.1). In addition, the reagent increases yields of the OptiPrep procedure by preserving the morphology and characteristics of the extracellular vesicles present in the original biofluid, leading to an overall enhancement of biomarker detection using OptiPrep gradient.

ExoMAX Opti Enhancer reagent comes in two sizes, 12 and 24 reactions*. With a simple 1:1 addition of ExoMAX reagent to your biofluid of interest, researchers can circumvent the time-consuming and laborious differential ultracentrifugation step of the OptiPrep gradient workflow. This is accomplished with overnight incubation of samples with ExoMAX Opti Enhancer, followed by next-day centrifugation at regular tabletop centrifuge speeds prior to loading onto the OptiPrep gradient.

***1 reaction is defined as treating 10ml of biofluid (e.g. cell culture media, serum/plasma brought up in buffer, etc.) with 10ml of ExoMAX Opti Enhancer reagent. Note, any volume of biofluid can be used for treatment using ExoMAX reagent at 1:1 ratio, providing increased sample scalability options.**

List of Components

Item	Volume	Storage Temperature
ExoMAX OptiPrep Enhancer Reagent (12 reactions)	120 mL	+4^{0}C or +25^{0}C
ExoMAX OptiPrep Enhancer Reagent (24 reactions)	240 mL	+4^{0}C or +25^{0}C

Storage

The ExoMAX Opti Enhancer reagents are shipped at RT or blue ice and should be **stored** at either +4°C or +25°C. Do not freeze. Properly stored kits are stable for 1 year from the date received.

Protocol for ExoMAX Opti Enhancer Reagent

1. Collect biofluid and centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.

2. Transfer supernatant to a sterile vessel and make note of the volume.

3. Add the appropriate volume of the reagent directly to the biofluid**

****Note: Small volume samples (e.g. serum/plasma) can be brought up to sufficient volume using 1X PBS or suitable buffers prior to addition of ExoMAX reagent.**

	Ratio (Reagent:Sample)	Sample Volume (ml)	Reagent Volume (ml)
ExoMAX Opti Enhancer	1:1	10	10

4. Mix well to combine by inverting the tube multiple times. Do not vortex.

5. Incubate samples overnight at 4^0C.

6. After incubation, centrifuge mixture at 1500 x g for 30 minutes. This may be performed at 4^0C or room temperature with similar results.

7. Aspirate the supernatant, being careful not to disturb the precipitated pellet.

8. Resuspend the pellet in the appropriate volume of PBS and layer it on top of an OptiPrep™ (Iodixanol) gradient.

Next Steps and Related Products

Application	Related Products	Website links
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck™ Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
Quantification of Exosomes		
Quantification of exosomes	FluoroCet assay	https://www.systembio.com/quantitate-exosomes/fluorocet
Quantification of exosomes	ExoELISA-ULTRA assay	https://www.systembio.com/quantitate-exosomes/exoelisa-ultra
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir™ kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

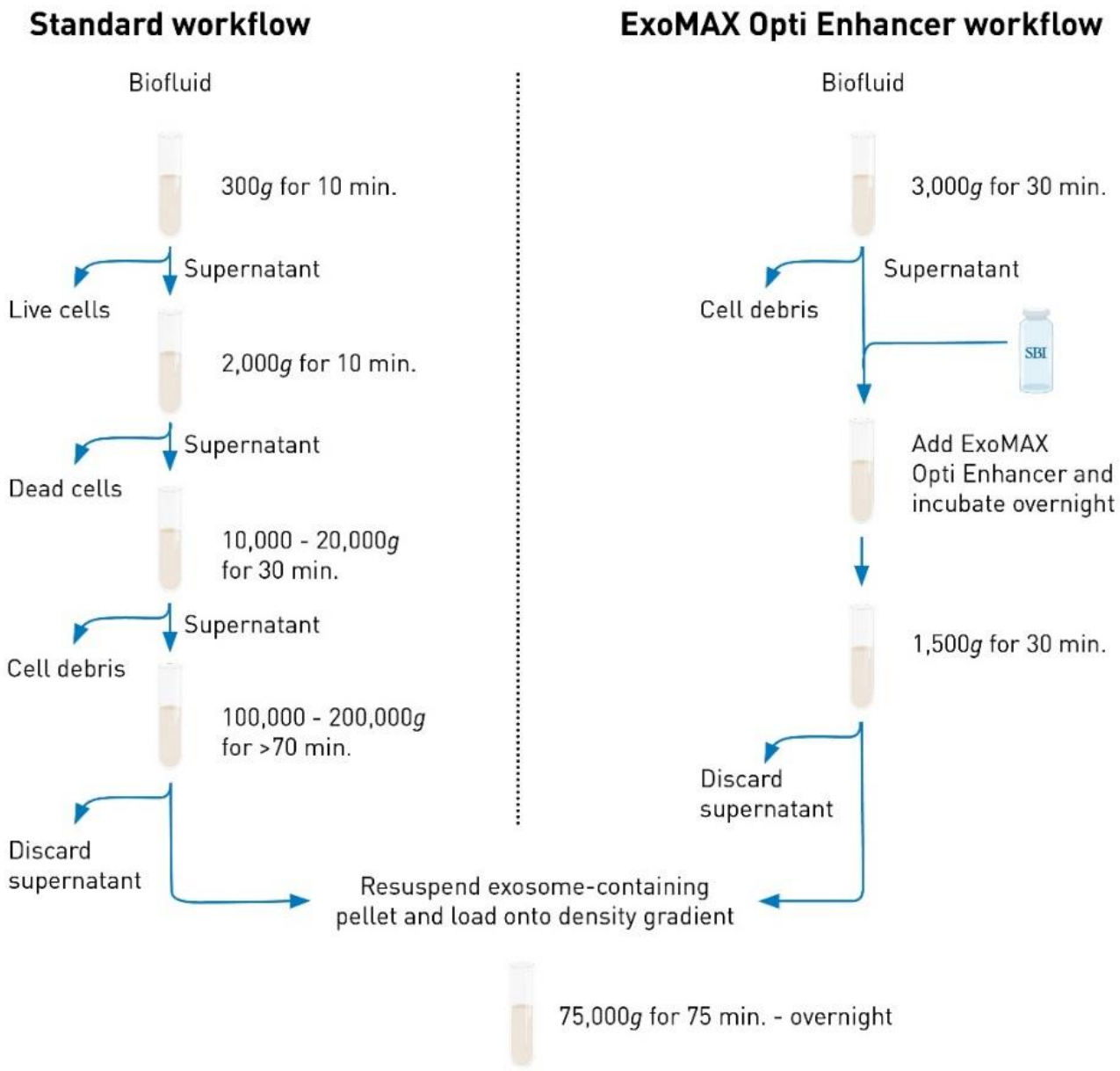

Figure 1. Workflow comparison for standard OptiPrep gradient vs ExoMAX Opti Enhancer for exosome isolation. The ExoMAX reagent replaces the initial differential ultracentrifugation step in the OptiPrep method.

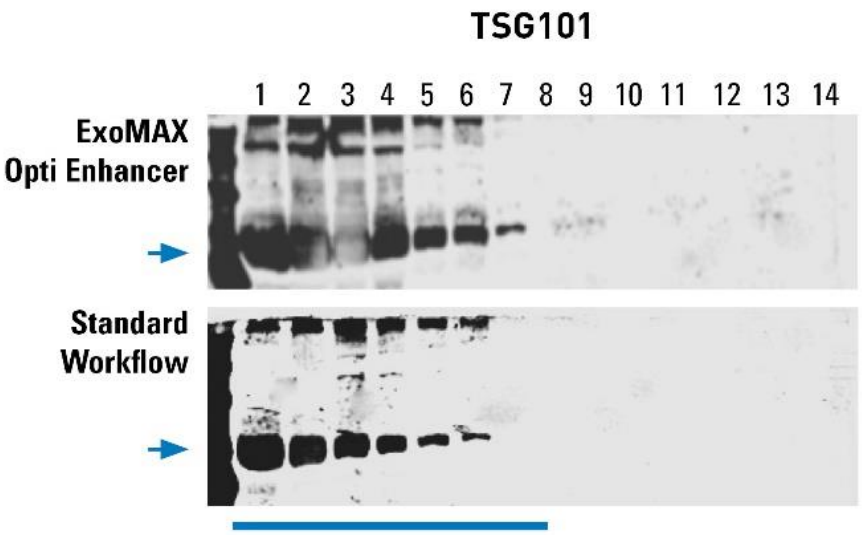

Figure 2. α-Tsg101 (a known exosomal marker) Western blot analysis of 14 individual fractions obtained from an OptiPrep gradient using ExoMAX Opti Enhancer or standard differential ultracentrifugation as a preparation step prior to loading onto the gradient. Banding intensity indicates higher yields for every fraction (1-7) using ExoMAX vs ultracentrifugation. Exosomes were isolated from 10ml of conditioned media from U1 cell

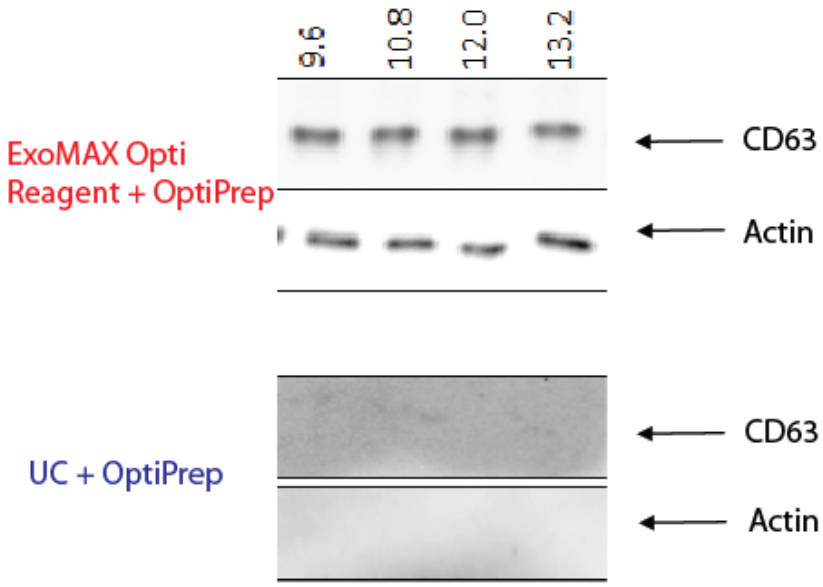

Figure 3. α-CD63 and beta-actin Western blot analysis of selected fractions obtained from an OptiPrep gradient using ExoMAX Opti Enhancer or standard differential ultracentrifugation as a preparation step prior to loading onto the gradient. CD63 and actin markers were not detected in UC+OptiPrep samples from same volume of sample processed (10ml of cell culture media)

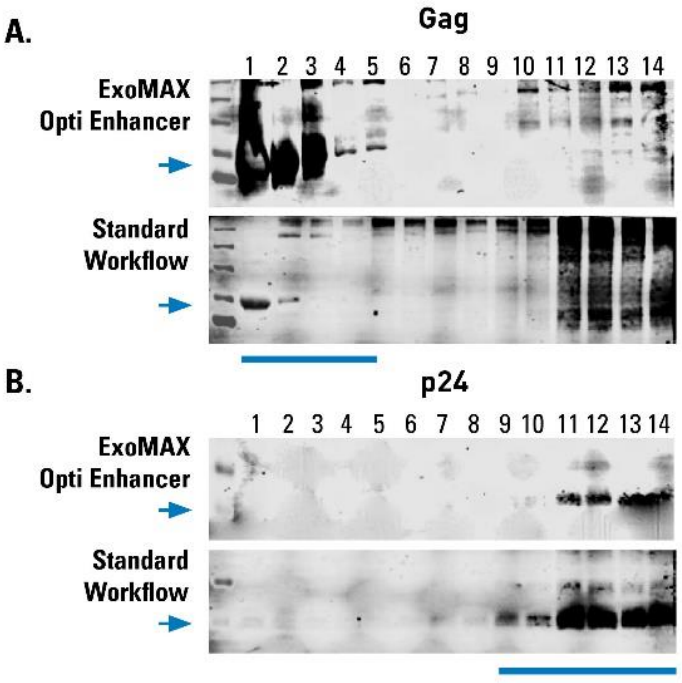

Figure 4. Exosomes prepared using ExoMAX Opti Enhancer can be purified away from virus. The HIV Gag protein is known to be abundant in exosomes from infected cells[1] whereas the p24 capsid protein is only found in assembled virus. (A) Density gradient fractions probed with anti-Gag antibody show the presence of Gag in the same fractions (fractions 1 – 5) that contain exosomes (exosome-containing fractions are identified in Figure 1). (B) However, HIV virus, as indicated by the presence of the HIV p24 capsid protein, is only detected in the non-exosome-containing fractions 11 – 13. *Data courtesy of Dr. Fatah Kashanchi, George Mason University.*

References

Narayanan A, *et al*. Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA. *J Biol Chem*. 2013; **288**(27):20014-33. PMCID: PMC3707700.

Technical Support

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Licensing and Warranty Statement

Limited Use License

Use of the ExoMAX Opti Enhancer reagent (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
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- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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