EXOSOME RESEARCH

ExoMAXTM OPTI ENHANCER

STREAMLINE DENSITY GRADIENT-BASED EXOSOME ISOLATION

SYSTEMBIO.COM/EXOMAX-OPTI-ENHANCER

HIGHLIGHTS

- High purity—supports separation of exosomes from viruses and protein aggregates
- High yield—delivers more exosomes than the traditional protocol
- More hands-free—three simple steps before the density gradient
- Flexible—compatible with downstream biomarker discovery and functional assays
- Scalable—pellet exosomes from any volume of conditioned media or biofluid and resuspend as needed for the density gradient

Easier preparation for density gradient ultracentrifugation

For researchers needing highly pure exosomes—such as exosomes separated from viruses—sucrose or OptiPrep™ (iodixanol) density gradient ultracentrifugation are the methods of choice. However, sample preparation prior to the density gradient is a time-consuming and multi-step process.

To streamline the pre-density gradient steps, SBI has developed ExoMAXTM Opti Enhancer, an easy-to-use reagent that can move samples to the density gradient in three easy steps (see back page for workflow comparison). Simply centrifuge the cell culture medium or body fluid to pellet cellular debris, incubate with ExoMAX Opti Enhancer, centrifuge again, and load the resuspended pellet onto the density gradient. The resulting exosomes harvested from the density gradient are present in higher amounts than when the standard preparation is used (Figure 1) and can be easily separated from viruses (Figure 2).

Use ExoMAX Opti Enhancer for a better density gradient ultracentrifugation-based exosome isolation workflow.

High-yield exosome isolation from HIV-infected T-cells

To demonstrate the excellent performance of ExoMAX Opti Enhancer, we isolated exosomes from HIV-infected T-cells. Equal volumes of conditioned medium from infected cells were collected and vesicles isolated using either ExoMAX Opti Enhancer reagent (Figures 1 and 2, top panels) or the standard protocol (Figures 1 and 2, bottom panels) before separation using OptiPrep gradient medium and ultracentrifugation at 110,000g for 70 minutes. Fractions from the gradient were collected and lysed for subsequent Western blot analysis, which show exosome yield (Figure 1), and separation of exosomes from HIV virus (Figure 2).

Figure 1. ExoMAX Opti Enhancer delivers high yields of exosomes.

Density gradient fractions probed with exosome-specific anti-Tsg101 antibody show exosomes in fractions 1 – 7, with higher exosome yields from the ExoMAX Opti Enhancer workflow.

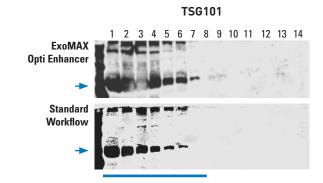
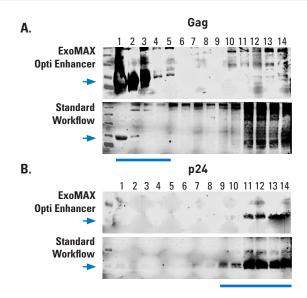




Figure 2. Exosomes prepared using ExoMAX Opti Enhancer can be purified away from virus.

The HIV Gag protein is known to be abundant in exosomes from infected cells¹ 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 that contain exosomes (1-5). **(B)** However, HIV virus, as indicated by the presence of the HIV p24 capsid protein, is detected in non-exosomecontaining fractions 11-13.



Building the tools that speed your research

With an eye on the latest advances, SBI finds promising technology and converts it into easy-to-use tools accessible to any researcher. Our growing exosome product portfolio is just one example. See what other ways SBI can drive your research forward—visit us at systembio.com.

ExoMAX Opti Enhancer Streamlines the Density Gradient Workflow

Standard workflow **ExoMAX Opti Enhancer workflow** Biofluid Biofluid 300g for 10 min. 3,000g for 30 min. Supernatant Supernatant Live cells Cell debris 2,000g for 10 min. Add ExoMAX Supernatant Opti Enhancer and Dead cells incubate overnight 10,000 - 20,000*q* for 30 min. Supernatant 1,500g for 30 min. Cell debris 100,000 - 200,000*q* for >70 min. Discard supernatant Discard supernatant Resuspend exosome-containing pellet and load onto density gradient 75,000g for 75 min. - overnight

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.

