A novel affinity-based method for the isolation of highly purified extracellular vesicles

Wataru Nakai, Takeshi Yoshida, Diego Diez, Yuji Miyatake, Takahiro Nishibu, Ryo Uekawa, Naoko Imawaka, Ken Naruse, Yoshihisa Sadamura & Rikinari Hanayama

Comparison of recovery yield and purity of sEVs

MagCapture™ Exosome Isolation Kit PS

Particle analysis of sEVs purified by the Tim4-affinity methods

Cell Culture Supernatant
Serum
Urine
Ca^2+

EDTA
Tim4 binds EV surface phosphatidylserine (PS) (Ca^2+ dependent)

Native Eulion of EV with Ca^2+ chelators (such as EDTA)

sEVs from K562 culture supernatant, human serum, or human urine were purified by the Tim4-affinity method or conventional methods. (Ultracentrifugation, Polymeric precipitation). Purified sEVs were analyzed by silver stain or Western blotting.

The sensitivity of the Tim4-based ELISA was 50 to 1,000 times higher than that of western blotting.

Western blotting vs Tim4-based ELISA

The linearity of dilution was good over the wide range of dilution of cell culture supernatant. Tim4-based ELISA Kit could detect sEVs included in the 0.34pg Culture Supernatant.

Linearity-of-Dilution Assessments

sEV standard: purified sEVs from K562 or COLO201 cell culture supernatant (sEVs were purified by the MagCapture™ Exosome Isolation Kit PS)

Sample: K562 or COLO201 cell culture supernatant

Primary Antibody: Anti-human CD63 Antibody

The standard curve of COLO201 was 1.0000, while the sample curve was 0.825.

Western blotting

Limit of Detection was about 0.34 pg or 0.34ng/mL

Limit of Detection (COLO201) was 3.3SD

PS Capture™ Exosome ELISA Kit

The linearity of dilution was good over the wide range of dilution of cell culture supernatant. Tim4-based ELISA Kit could detect sEVs included in the 0.34pg Culture Supernatant.

Linearity-of-Dilution Assessments

sEV standard: purified sEVs from K562 or COLO201 Culture supernatant (sEVs were purified by the MagCapture™ Exosome Isolation Kit PS)

Sample: K562 or COLO201 cell culture supernatant

Primary Antibody: Anti-human CD63 Antibody

The standard curve of COLO201 was 1.0000, while the sample curve was 0.825.

Western blottting

Limit of Detection was about 0.34 pg or 0.34ng/mL

Limit of Detection (COLO201) was 3.3SD

MagCapture™ Exosome Isolation Kit PS

Particle analysis of sEVs purified by the Tim4-affinity methods

The appearance of sEVs isolated by the Tim4-affinity method matched the typical saucer shape as previously reported.

The mean size of sEVs purified by the conventional methods was larger than that by the Tim4-affinity method due to increased populations of aggregated or fused sEVs larger than 200nm.

Tim4-affinity method could isolate sEVs with higher quality than that by using conventional methods.

Comparison of recovery yield and purity of sEVs

The Uptake of sEV

Microarray analysis of miRNA

Conclusion

sEVs from K562 cells were labeled by the fluorescence dye and the uptake of these sEVs by HeLa cells was examined.

sEVs purified by the Tim4-affinity method were more efficiently incorporated into the HeLa cells than that by the ultracentrifugation.

sEVs from K562 cells were examined by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.

MagCapture™ Exosome Isolation Kit PS

Sample Type: cell culture supernatant, serum, plasma, urine, etc.

MagCapture™ Exosome Isolation Kit PS can purify any EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that human, mouse, and bovine EVs can be purified by this isolation kit.

Tim4-affinity is an ideal method to isolate sEVs containing exosome with much higher purity than that by using other conventional methods.

Tim4-affinity method is suitable for isolating sEVs from biofluids including serum and urine.

sEVs from K562 cells were examined by transmission electron microscope (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.

The appearance of sEVs isolated by the Tim4-affinity method matched the typical saucer shape as previously reported.

The mean size of sEVs purified by the conventional methods was larger than that by the Tim4-affinity method due to increased populations of aggregated or fused sEVs larger than 200nm.

Tim4-affinity method could isolate sEVs with higher quality than that by using conventional methods.

Comparison of recovery yield and purity of sEVs

The Uptake of sEV

Microarray analysis of miRNA

Conclusion

sEVs from K562 cells were examined by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.

MagCapture™ Exosome Isolation Kit PS

Sample Type: cell culture supernatant, serum, plasma, urine, etc.

MagCapture™ Exosome Isolation Kit PS can purify any EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that human, mouse, and bovine EVs can be purified by this isolation kit.

Tim4-affinity is an ideal method to isolate sEVs containing exosome with much higher purity than that by using other conventional methods.

Tim4-affinity method is suitable for isolating sEVs from biofluids including serum and urine.

sEVs from K562 cells were examined by transmission electron microscope (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.

The appearance of sEVs isolated by the Tim4-affinity method matched the typical saucer shape as previously reported.

The mean size of sEVs purified by the conventional methods was larger than that by the Tim4-affinity method due to increased populations of aggregated or fused sEVs larger than 200nm.

Tim4-affinity method could isolate sEVs with higher quality than that by using conventional methods.

Comparison of recovery yield and purity of sEVs

The Uptake of sEV

Microarray analysis of miRNA

Conclusion

sEVs from K562 cells were examined by transmission electron microscope (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.

MagCapture™ Exosome Isolation Kit PS

Sample Type: cell culture supernatant, serum, plasma, urine, etc.

MagCapture™ Exosome Isolation Kit PS can purify any EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that human, mouse, and bovine EVs can be purified by this isolation kit.

Tim4-affinity is an ideal method to isolate sEVs containing exosome with much higher purity than that by using other conventional methods.

Tim4-affinity method is suitable for isolating sEVs from biofluids including serum and urine.

sEVs from K562 cells were examined by transmission electron microscope (TEM) and nanoparticle tracking analysis (NTA) using NanoSight.