# Magnetic beads for fast and reproducible isolation/characterization of exosomes based on surface protein expression

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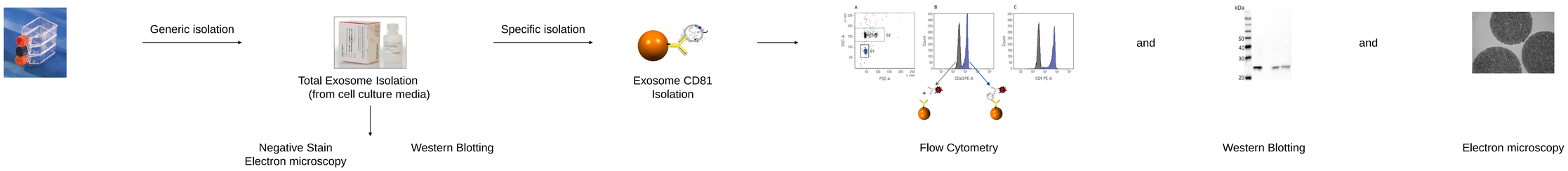
## INTRODUCTION

Exosomes are small (30-120 nm) vesicles containing proteins, RNA and lipids, secreted by all cell types in culture and found in most body fluids. There is hope that miRNAs in circulating exosomes provides a screening tool for very early detection of cancer. The established standard for exosome isolation is ultracentrifugation *(Théry et al, 2006)* . However, this method can not discriminate between exosomes subpopulations and other microvesicles. Dynabeads serve as versatile tools for exosome isolation and characterization and can be tailored for isolation of cell specific exosomes.




## RESULTS – TEM analysis on generic exosome populations

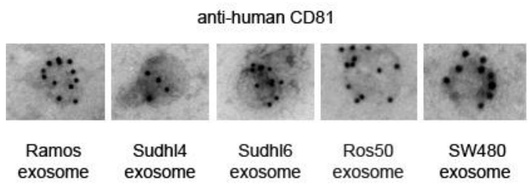



**Figure 1.** Total exosome isolation preparations were characterized by immunolabelling and negative stain electron microscopy. The generic exosomes were prepared from B-cell lymphoma cell lines (Ramos, Sudhl4, Sudhl6 and Ros50) and SW480 cell culture supernatants. Prior to microscopy the exosomes were labelled for the tetraspanin protein CD81, commonly identified in exosomal preparations. Ultrastructural analysis demonstrated intact exosome like structures in all preparations. The size of the structures was in the range of 50-100nm and labelled for CD81. These observations demonstrate that we have exosome like structures in our total exosome isolation preparations and that they label for a typical exosomal marker (CD81)

## RESULTS – WB analysis on generic exosome populations and CD81 positive sub-populations

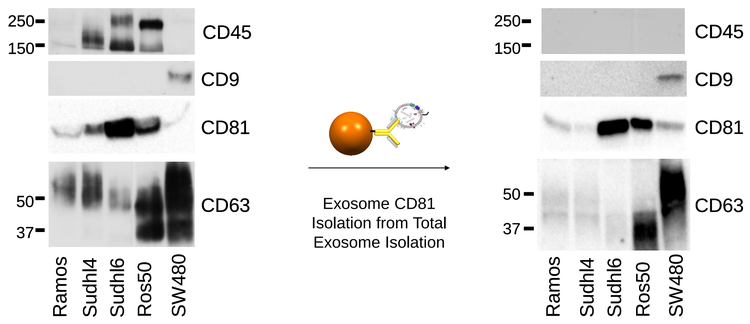



**Figure 2.** Total exosome isolation preparations were characterized by western blot analysis. The blots were labelled for typical exosomal markers (CD9, CD81 and CD63) in addition to CD45. CD63 was detected in exosome lysates from all 5 exosome sources. The amount of CD63 seems to vary in addition to the glycosylation pattern. Exosomes derived from Ramos, Sudhl4 and Sudhl6 contained significantly less CD63 compared to Ros50 and SW480 derived exosomes. Exosomes derived from B-cell lymphoma cell lines contained CD81, however, in various amounts. CD9 was only observed in exosomes derived from SW480 and could not be detected in any of the B-cell lymphoma derived exosomes. In terms of B-cell markers, CD45 was observed in Sudhl4, Sudhl6 and Ros50 and was absent in Ramos and SW480 derived exosomes. CD81 positive sub-populations of exosomes were then analysed after isolation from total exosome isolations derived from SW480, Sudhl4, Sudhl6, Ramos and Ros50 cell lines. The isolated exosomes were then processed for western blotting and labelled for typical exosome markers such as CD63, CD81, CD9 and CD45. In terms of CD63, CD81 and CD9 labelling the pattern was similar to the labelling observed in the total exosome isolation preparations. Interestingly, CD45 can not be detected.



## RESULTS – TEM analysis on exosome sub-population

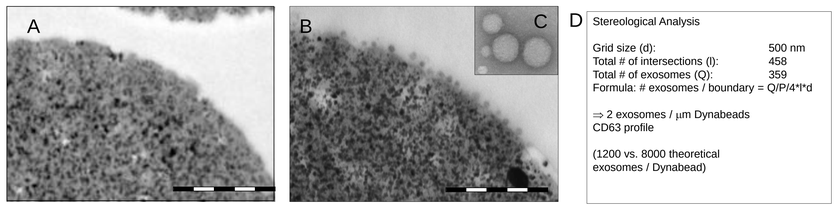



**Figure 3.** Ultrastructural analysis of ultra thin sections of Dynabeads CD63 after exosome isolation revealed exosome-like structures in the size range of 100nm. Morphologically, the structures appeared electron dense, spherical and evenly distributed at the surface of the beads. (A and B) Transmission Electron microscopy (TEM) of exosomes isolated with Exosome CD63 Isolation using low (A) and high (B) concentration of exosomes. Samples prepared according to *Pedersen et al 1999*. Bar 500nm. C) Negative Stain TEM of exosomes prior to Exosome CD63 Isolation. Samples were prepared according to *Pedersen et al 2000*. D) Stereological analysis of exosome density on the bead surface.

## RESULTS – Flow analysis on exosome sub-populations

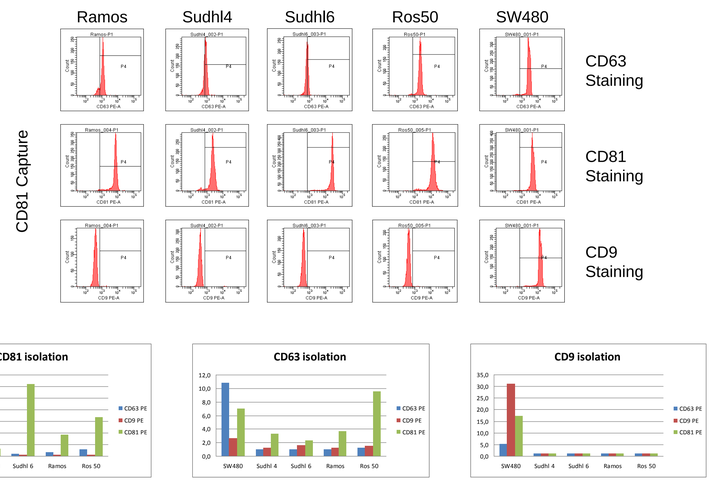



**Figure. 4.** In these experiments we have used Dynabeads coated with anti-human CD63, anti-human CD81 and anti-human CD9 in order to isolate CD63 positive, CD81 positive and CD9 positive sub-populations of exosomes from total exosome isolations derived from SW480, Sudhl4, Sudhl6, Ramos and Ros50 cell lines. The isolated exosomes were stained for typical exosome markers such as CD63, CD81 and CD9. Exosomes isolated with CD81 showed that all B-cell lymphoma derived exosomes expressed CD81, however at different levels. Furthermore, CD81 isolated exosomes derived from Ros50 cells also expressed some CD63. Interestingly, non of the CD81 isolated B-cell lymphoma derived exosomes isolated exosomes expressed CD9. This was supported by lack of exosome isolations using Dynabeads CD9. Only SW480 derived exosomes expressed all 3 markers. Isolation of CD63 positive exosomes from all cell lines showed CD81 expression at different levels. Only SW480 derived exosomes showed very high level of CD63 expression, while B-cell lymphoma derived exosomes show low CD63 expression.

## RESULTS – Flow analysis method qualification

| Qualification parameter | Qualification results:<br>Signal to Noice (S/N) |
|---|---|
| Specificity | Specificity of relevant beads:<br>S/N: 20 x irrelevant beads |
| Presition (repeatablility) | Average (n=15): S/N = 19,9<br>Range (n=15): S/N: 19,5 – 21,5<br>SD (n=15): 0.9<br>% RSD: 4,6 |
| Intermediate presition | Grand average (n=45): S/N = 18,9<br>Grand SD (n=45): 2,7<br>Grand % RSD: 14,4 |
| 95% confidence limits | S/N: 13,5 – 24,2 |
| Linearity | $R^2$ = 0,9688 |
| LOD | Median value: 330 MFI |
| LOQ | Limit of quantification: 353 MFI |

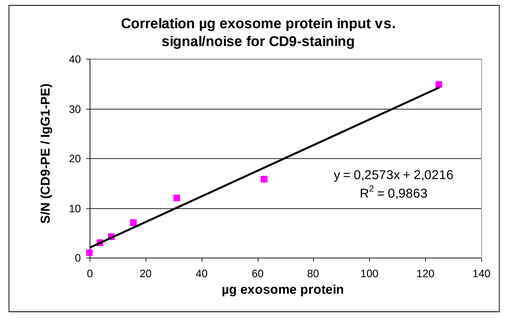



**Figure 5.** Flow analysis method for detection of bead bound exosomes was qualified for specificity, repeatability, intermediate precision, limit of detection (LOD) and limit of quantification (LOQ) using signal to noise (S/N) as readout. Intermediate precision included analysis performed on 3 days and by 3 operators.

## CONCLUSION

- We have demonstrated the use of Dynabeads for isolation/analysis of exosomes sub-populations from pre-enriched cell culture supernatant using flow cytometry, electron microscopy and western blotting.
- We have demonstrated that exosomes derived from various cancer related cell lines produce exosomes with different identity based on surface expression markers (CD9, CD63, CD81)
- We have developed a flow analysis assay for exosomes with high reproducibility indicating that Dynabeads are suitable for screening of exosome content in patient samples..

## REFERENCES

Théry et al (2006) Current Protocols in Cell Biology 3.22.1-3.22.29
Pedersen et al (1999) J of Virol, p. 2016–2026 Vol. 73
Pedersen et al (2000) J of Virol, p. 3525-3536 Vol. 74