Application Note

Exosome marker detection: an exquisitely sensitive Europium Time Resolved Immunofluorescence assay

Exosome detection from cell culture media using ExoLISA™ assay and BMG LABTECH TRF microplate readers

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- **Experiment**
  Detect exosome markers using ExoLISA™ assay combined with BMG LABTECH plate readers
- **Exosomes origin**
  25 µg exosomes isolated from conditioned cell culture media (prostate and colorectal cell lines) using Exo-spin™ exosome purification kit
- **Concentration range**
  Serial dilutions in PBS from 100 µg/ml to 0.8 µg/ml, obtaining a total of 8 different exosome concentrations
- **Volume used per well**
  100 µl of each dilution

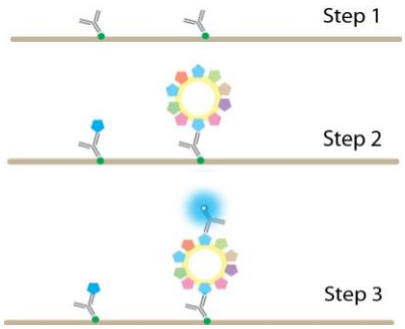
Summary

The rapidly expanding EV research field benefits tremendously from technologies which enable the detection of exosomes and their content. Tetraspanin proteins such as CD9, CD63, and CD81 with exposed domains are particularly enriched in the exosome membrane and are often used as exosome biomarkers. ExoLISA™ - the Time Resolved Immunofluorescence exosome detection assay - has been developed specifically for analyzing the levels of these tetraspanin proteins on the surface of exosomes. ExoLISA™ assay enables researchers to directly measure tetraspanins present on exosomes from sources such as cell culture media, urine, or blood with low background signals and high sensitivity.

The assay has been successfully evaluated on several BMG LABTECH plate readers, including FLUOstar Omega, CLARIOstar Plus and the PHERAstar FSX. Accurate and reliable TRF signals were obtained for different exosome markers, with all instruments meeting the sensitivity requirements, ensuring optimal assay performance. Consistently linear signals were generated over a wide range of exosome concentrations, demonstrating the robustness of the assay. The ExoLISA™ assay can also be used with unpurified samples.

- **Detects different expression levels** of exosome markers
- **Simple** assay offering a high degree of reproducibility
- **Highly sensitive** and specific: only antigens displayed in multiple copies are detectable

Methods



Step 1: Biotinylated antibody is bound to streptavidin coated assay plates. The kit provides a 96-well microplate with an 8-well strip format.

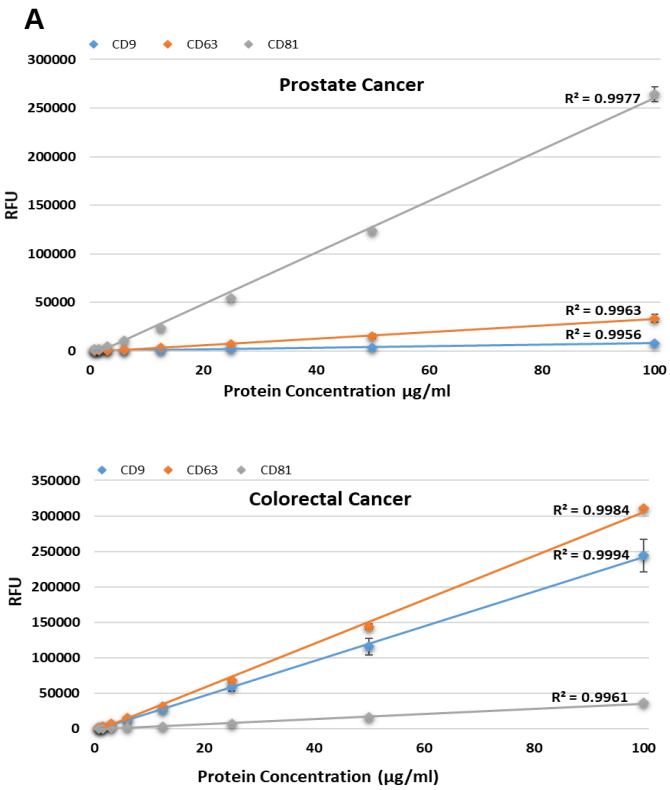
Step 2: Biological samples containing exosomes are added to the plate. Exosomes bearing the appropriate antigen (CD9, CD63, or CD81) and any free antigen are captured by the immobilized antibody.

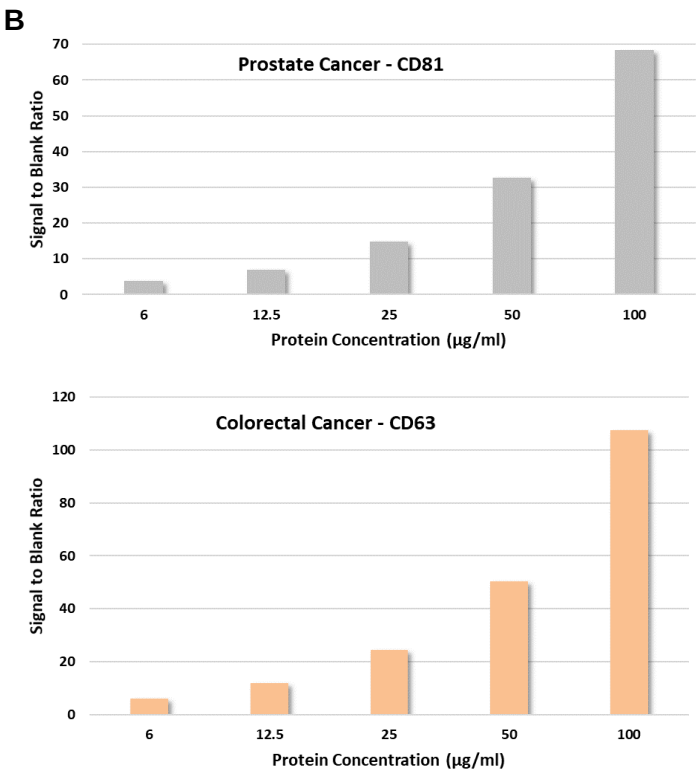
Step 3: Europium-labeled antibody is added and binds specifically to exosome antigens. Free antigen is not bound by the europium-labeled antibody (since the epitope is already occupied by the immobilized antibody) and is therefore not detected. Samples are then read on a Time-Resolved Fluorescence (TRF) plate reader such as the PHERAstar FSX.

Table 1. Instrument settings

Optic settings	Time resolved fluorescence (TRF)	
	Filters	Excitation: 337 nm Emission: 615 nm
General settings	Number of flashes	200 with flash lamp
	Settling time	0.3 s
	Integration start	200 µs
	Integration time	400 µs

Results





(A) Marker detection on exosomes from prostate and colorectal cancer cells, respectively. Each datapoint represents an average value (N=3). The error bars for each datapoint are very small, indicating extremely little variance in signal output. Moreover, the R^2 values for each serial dilution demonstrate excellent linearity. This is consistent with the data obtained for total protein concentration.

(B) Signal to blank ratio of CD81/CD63 detection on exosomes from prostate and colorectal cancer cells, respectively. Each datapoint represents an average value (N=3). The data presented further demonstrates the consistency of the TRF signal generated by the assay over a wide range of concentrations.

References

Marina Colombo *et al.* Annu. Rev. Cell Dev. Biol. (2014) 30: 255-89. DOI: 10.1146/annurev-cellbio-101512-122326
Diedrik Duijvesz *et al.* Int. J. Cancer (2015) 137: 2869-78. DOI: 10.1002/ijc.29664
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