Exosomes are released by a variety of cell types either constitutively or in a stimulation-induced fashion. Depending on the originating cell, exosomes are loaded with a specific set of proteins, lipids, and nucleic acids. To investigate the origin, composition, and function of exosomes in biological fluids (e.g., plasma), specific markers are needed. We established a multiplex bead-based platform consisting of capture and detection antibodies to analyze the composition of exosome surface proteins in a given sample by flow cytometry.

### Methods

**Capture antibody beads**

- **Up to 30 capture antibody beads**

**Incubation over night, washing**

1 h incubation, washing

**Flow cytometry analysis**

### Results

#### 1 Signal intensities are specific and not impaired by the number of beads or the composition of the bead set

In order to verify the specificity of exosome binding to the capture antibody beads, the beads were incubated with NK cell exosomes alone or in combination with soluble mouse IgG1 as isotype control or soluble CD63 antibody to block the binding to the anti-CD63 beads. In contrast to the isotype control, blocking with soluble CD63 antibody specifically inhibited binding of the exosomes to the anti-CD63 beads (fig. 3A), demonstrating that exosome binding to the beads was specific. Signal intensities of the capture antibody beads were comparable in an 8-plex and 34-plex format (fig. 3B), suggesting that the composition of the bead set or the number of beads used did not affect the signal intensities.

### Conclusion & outlook

- The multiplex bead platform allows the specific detection of exosome surface proteins and the assessment of their relative abundance on exosomes from different sources.
- The composition of the multiplex bead set did not affect signal intensities.
- The common exosome markers were not equally distributed in all exosome populations: NK cell exosomes had less CD9, while CD31 was underrepresented on platelet exosomes.
- Well-established marker cells were detectable on the secreted exosomes, i.e., CD3 on T cell exosomes, CD56 on NK cell exosomes, and CD61 on platelet exosomes.
- The presence of HLA class II and CD61 on plasma exosomes suggests that HLA class II-expressing cells, such as antigen-presenting cells, and platelets were the main sources of exosomes in peripheral blood.

### References

4. Miltenyi Biotec GmbH, Department of Research & Development, Bergisch Gladbach, Germany

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**A multiplex bead platform for the characterization of cell culture–and plasma-derived exosomes**

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