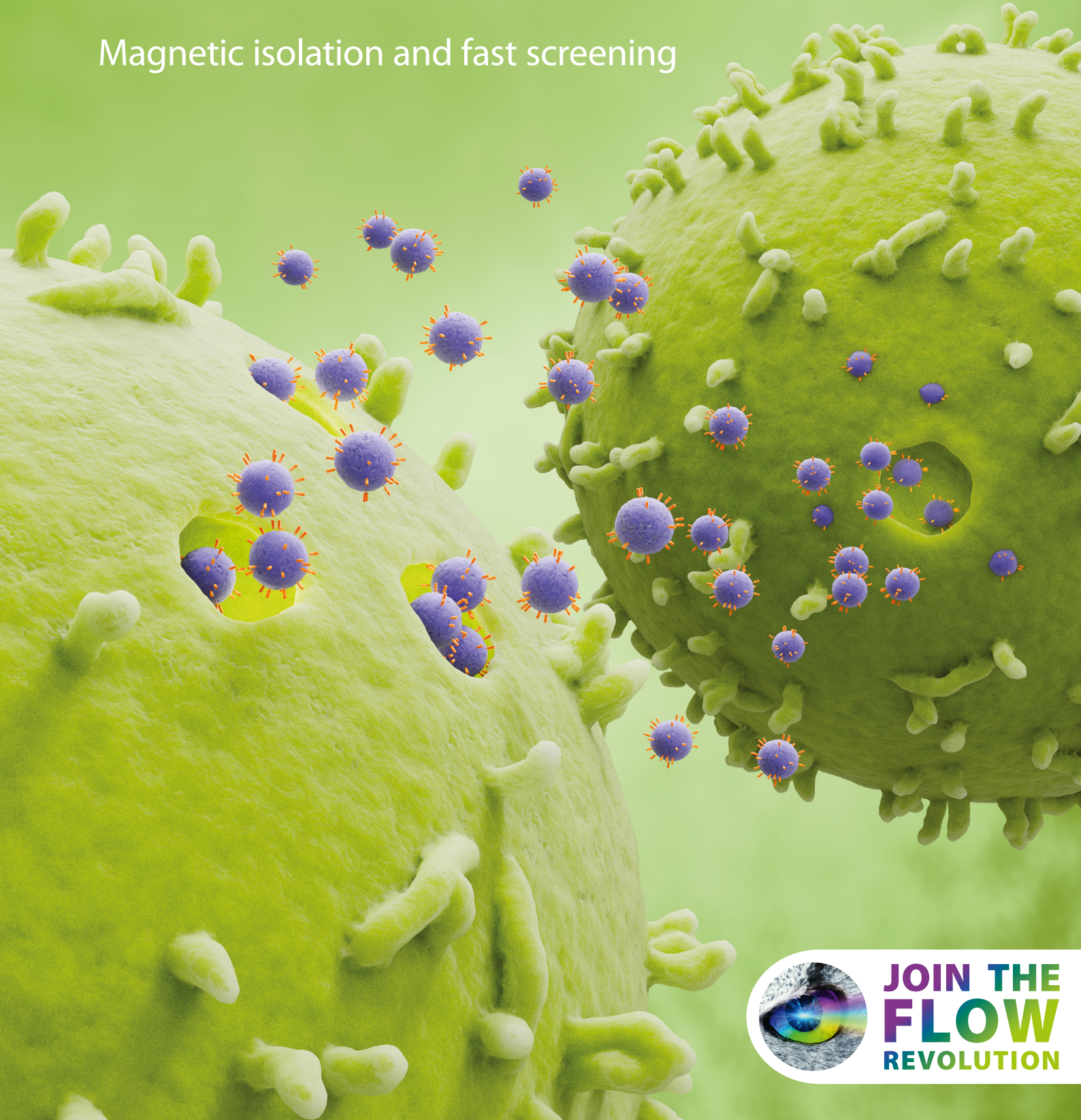




Miltenyi Biotec

Exosome research solutions

Magnetic isolation and fast screening



**JOIN THE
FLOW
REVOLUTION**

Isolating exosomes the easy way

Without ultracentrifugation

Isolation of extracellular vesicles from cell culture supernatant or body fluids

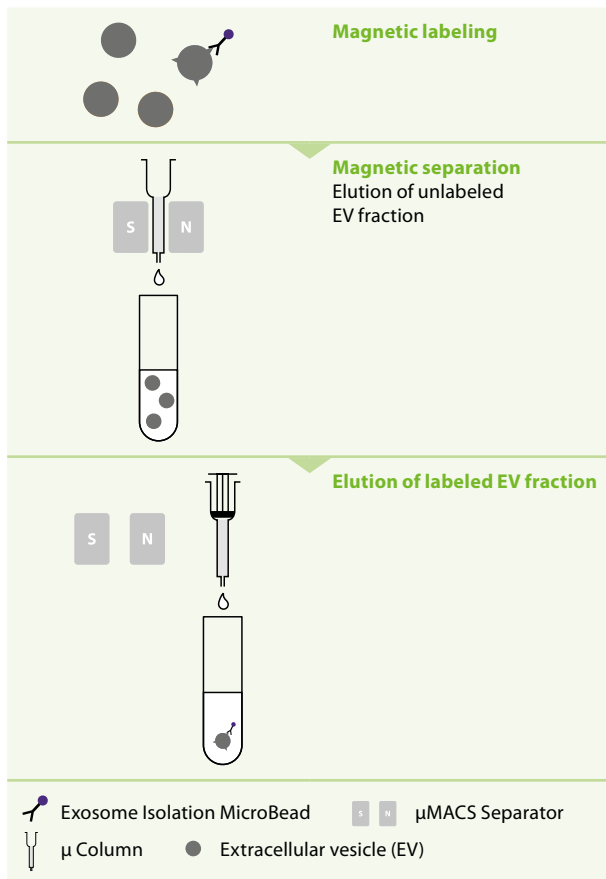


Figure 1: Principle of magnetic isolation of EVs using an Exosome Isolation Kit.

Exosomes – small vesicles, big impact

Exosomes are extracellular vesicles (EVs) of endocytic origin, released by numerous cell types including immune cells, stem cells, and neurons. They carry various molecules and play an important role as intercellular messengers in health and disease. There is growing interest in applying exosomes in clinical settings as they can potentially be used as diagnostic biomarkers and therapeutic agents.

MACS® Technology – the proven technique for magnetic separation

EVs can be easily isolated by MACS® Technology – the tried and tested cell isolation method cited in tens of thousands of publications. This technique owes its longstanding success to the combination of three components: i) nano-sized superparamagnetic MACS MicroBeads coupled to antibodies specifically detecting certain epitopes on the surface of cells or EVs, ii) MACS Separators, and iii) MACS Columns generating a strong magnetic field.

Miltenyi Biotec offers dedicated kits for isolating EVs based on the tetraspanin proteins CD9, CD63, and CD81, which are known to be present on the surface of exosomes. Exosome Starting Kits contain all materials required for convenient exosome isolation, including separator and columns.

Isolation of EVs in three easy steps

First, EVs contained in cell culture supernatant or body fluids are magnetically labeled with Exosome Isolation MicroBeads CD9, CD63, or CD81. The labeled EVs are then loaded onto a μ Column, which is placed in the magnetic field of a μMACS™ Separator. Magnetically labeled EVs are retained within the column, while unlabeled vesicles and cell components run through. After removing the column from the magnetic field, the intact EVs can be eluted (fig. 1).

- Fast (< 2h) and easy isolation of exosomes without ultracentrifugation
- Targeted isolation based on CD9, CD63, or CD81, or all three markers combined
- EV isolation from cell culture supernatant or body fluids like plasma, urine, or ascites

New horizons for exosome analysis

Fast screening by flow cytometry

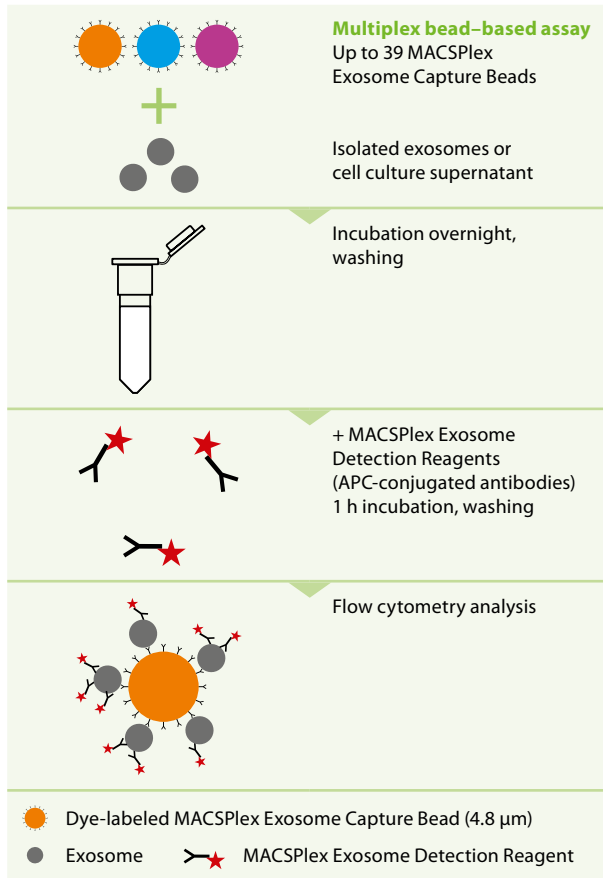


Figure 2: Principle of the MACSPlex Exosome Kit. Isolated exosomes are incubated overnight with 39 differently labeled MACSPlex Exosome Capture Beads each coupled to a different antibody. Exosomes bound to the beads are detected with MACSPlex Exosome Detection Reagents by flow cytometry.

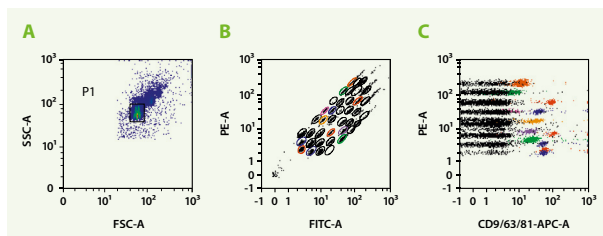


Figure 3: Exosome analysis based on the MACSPlex Exosome Kit. The example shows (A) gating according to bead size, (B) discrimination of differently labeled bead populations, and (C) measurement of signal intensities of the single bead populations.

MACSPlex Exosome Kit – the solution for comprehensive analysis

Due to their small size, it has been difficult thus far to analyze EVs by standard flow cytometry, which hampered scientific advancement in this field. To facilitate comprehensive EV analysis, Miltenyi Biotec developed the MACSPlex Exosome Kit. This novel tool enables an easy and fast screening of potential EV surface proteins.

- Unique multiplex bead platform for protein profiling of EVs by flow cytometry
- Saves precious sample material by screening 37 surface markers simultaneously
- Analysis of EVs from cell culture supernatant or body fluids

Principle of the MACSPlex Exosome Kit

The MACSPlex Exosome Kit allows for the simultaneous detection of 37 surface epitopes that are known to be present on different EVs. The kit is based on a cocktail of various fluorescently labeled bead populations, the MACSPlex Exosome Capture Beads, which can be distinguished by flow cytometry.

Each of these MACSPlex Exosome Capture Bead populations is coupled to a specific antibody binding to a respective exosomal surface epitope. Exosomes bound to the MACSPlex Capture Beads are stained with MACSPlex Exosome Detection Reagents, i.e., a cocktail of APC-conjugated antibodies against the tetraspanins CD9, CD63, and CD81. This leads to the formation of complexes, each consisting of i) MACSPlex Exosome Capture Bead, ii) exosome, and iii) APC-conjugated antibodies (fig. 2). These complexes can then be analyzed based on the fluorescence characteristics of both the MACSPlex Exosome Capture Beads and the APC-conjugated antibodies (fig. 3). Two isotype control beads are included in the kit to check for non-specific binding.

Exosome Isolation Kits

For efficient downstream analysis



Fast and reliable EV isolation and protein profiling

EVs isolated with Exosome Isolation Kits based on single tetraspanins, i.e., CD9, CD63, or CD81, can be easily analyzed for their protein profiles using the MACSPlex Exosome Kit (fig. 4). For most of the MACSPlex Exosome Capture Bead types, EVs isolated based on CD63 gave the strongest signals, followed by CD81 and CD9. EVs are traditionally prepared by ultracentrifugation. However, this method is time consuming and can lead to inconclusive results in protein profiling experiments (fig. 4): EVs isolated by ultracentrifugation showed only weak fluorescence signals compared to EVs isolated by MACS® Technology (table 1), when analyzed with the MACSPlex Exosome Kit (table 3).

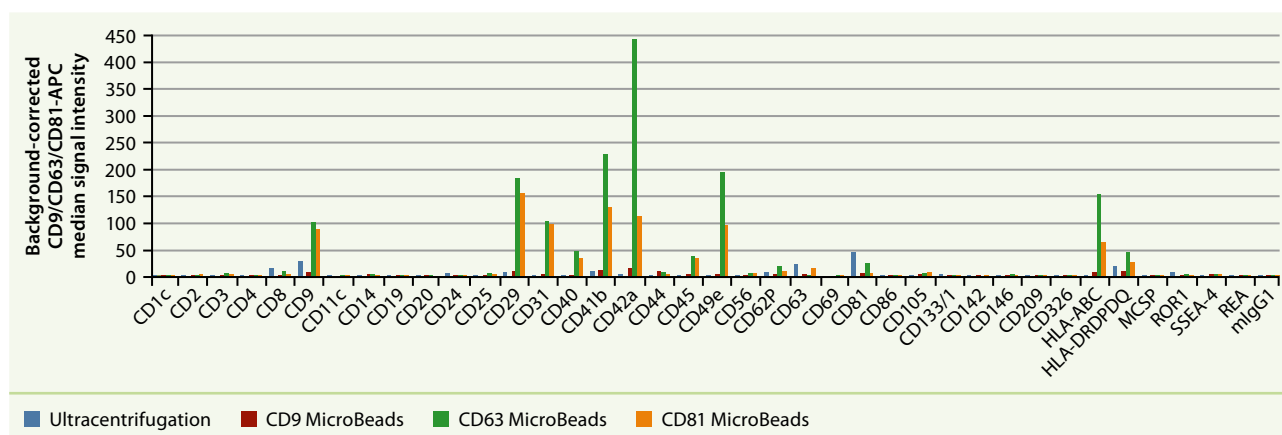


Figure 4: Surface marker profiles of EVs isolated from plasma by ultracentrifugation or immunomagnetic isolation using the Exosome Isolation Kits CD9, CD63, or CD81. EVs from 2 mL of plasma were isolated using one of the kits or by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL, and exosomes were analyzed using the MACSPlex Exosome Kit. Data indicate median APC signal intensities of isolated EVs incubated with the 39 MACSPlex Exosome Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

Product	Order no.
Exosome Isolation Kit CD9, human	130-110-913
Exosome Isolation Kit CD63, human	130-110-918
Exosome Isolation Kit CD81, human	130-110-914
Exosome Isolation Kit Pan, human	130-110-912

Table 1: Products for isolating EVs.

Product	Order no.
Exosome Isolation Kit CD9, mouse	130-117-042
Exosome Isolation Kit CD63, mouse	130-117-041
Exosome Isolation Kit CD81, mouse	130-117-040
Exosome Isolation Kit Pan, mouse	130-117-039

Exosome Starting kits including μ MACS Separator and MultiStand, are also available for all three markers.

MACSPlex Exosome Kit

For comprehensive EV profiling

Antibodies for EV analysis		
HLA-ABC	CD19	CD62P
HLA-DR, DP, DQ	CD20	CD63
MCSP	CD24	CD69
ROR1	CD25	CD81
SSEA-4	CD29	CD86
CD1c	CD31	CD105
CD2	CD40	CD133/1
CD3	CD41b	CD142
CD4	CD42a	CD146
CD8	CD44	CD209
CD9	CD45	CD326
CD11c	CD49e	Mouse IgG1 Control
CD14	CD56	REA Control

Table 2: Overview of surface marker and control antibodies used for EV analysis by the MACSPlex Exosome Kit, human.

Product	Order no.
MACSPlex Exosome Kit, human	130-108-813
MACSPlex Exosome Kit, mouse	130-122-213

Table 3: Products for characterizing surface markers on EVs by flow cytometry.

EVs from plasma samples

The surface signature of EVs has been the focus of many studies to better understand the heterogenous nature of EVs, identify potential EV subsets with therapeutic activity and reveal disease-specific biomarkers. However, protein profiling of EVs requires a reliable and robust method for the analysis of multiple markers.

The MACSPlex Exosome Kits are the ideal solution enabling the screening of up to 37 markers in parallel by flow cytometry from cell culture supernatant or body fluid-derived EVs.

Figure 5 shows a protein profile of plasma-derived EVs from one donor at different input doses. Many markers that are expressed on specific blood cell types, like CD24 (B cells) or CD8 (T cells), are also present on plasma-derived EVs. As expected, signal intensities for all positively stained bead populations decreases with decreasing input. Therefore, the expression of EV surface markers can be detected in a robust, sensitive, and reproducible way. Table 2 provides an overview of the antibodies used for EV analysis.

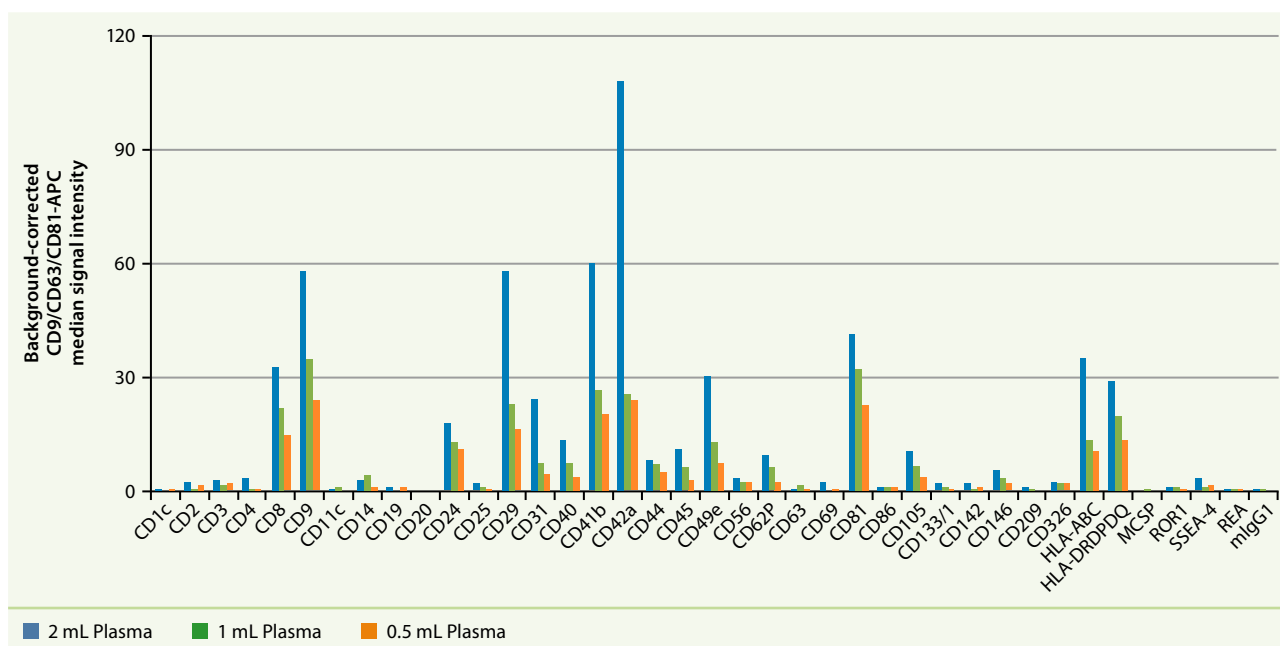


Figure 5: Characterization of plasma-derived EVs with the MACSPlex Exosome Kit, human. Plasma EVs from one donor were isolated with the Exosome Isolation Kit CD63, human. Different volumes of plasma were used. Isolated plasma EVs were analyzed with the MACSPlex Exosome Kit, human. Data indicate median APC signal intensities of plasma EVs incubated with the 39 MACSPlex Exosome Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.



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