Flow Post-its
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FMO Controls

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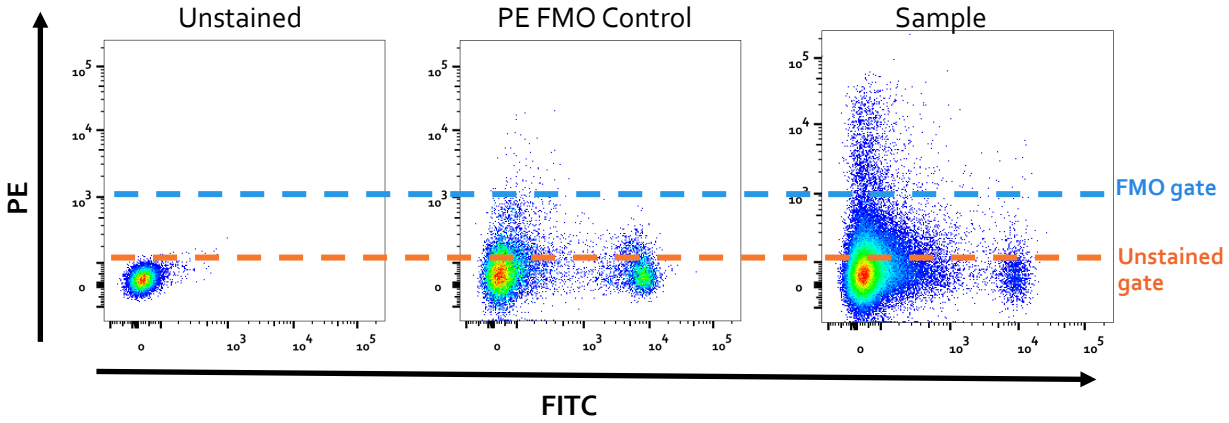
In multicolor Flow Cytometry experiments, spillover spreading is seen across detectors. This spread is fluorochrome dependedent and can impact resolution, which cannot be improved by changing the voltages or gains. In multicolor panels where spreading is seen, **F**luorescence **M**inus **O**ne (FMO) controls become essential to set appropriate gates, particularly when the expression is dim or a smear



As illustrated to the left, establishing the cut-off between positive and negative populations using the unstained control is not as accurate as using the FMO.

The FMO control allows us to account for the additive effect of spreading from multiple fluorochromes into the detector of interest, resulting in a more precise gating of positivity.

Fluorochrome	Unstained	FMO Control	Sample
APC	-	✔	✔
APC-Cy7	-	✔	✔
FITC	-	✔	✔
PE	-	-	✔
PE-Cy7	-	✔	✔
PerCP-Cy5.5	-	✔	✔

Helpful Hints

- FMO controls are the **same cells** as in the experiment **stained with all the fluorochromes in the panel except one**.
- Beads cannot be used for FMO controls.
- FMO controls **do not assess non-specific binding** of antibodies Antibody titration is still necessary.
- The use of isotype controls to define positivity is not correct. FMOs and other appropriate controls are recommended.
- Spreading will differ depending on fluorochrome combinations. Panels should be designed accordingly to reduce spread.