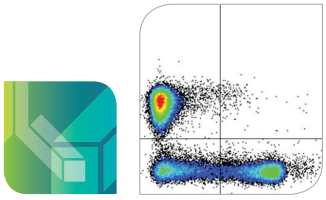
Take Control

Crucial controls for flow cytometry



Controls are vital in any experiment to reliably distinguish your results from background variation and non-specific effects. This handy checklist briefly describes essential controls for flow cytometry that you must consider to ensure publication quality flow cytometry data.

1

Unstained control

The first thing to identify in flow cytometry is your cell population by its forward and side scatter characteristics. After this you need to determine where the negative population will be. To do this you should always have an unstained population. This will allow you to determine the level of background fluorescence or autofluorescence and set your voltages and negative gates appropriately.

2

Isotype controls

The use of isotype controls in flow cytometry is controversial and often divides researchers. Isotype controls have been developed for surface staining and their role is to ensure the observed staining is due to specific antibody binding to the target rather than an artifact or Fc receptor binding. Ensure you are using the right isotype control with the correct fluorophore and always consider what additional controls you may need to do.

3

Single staining and compensation controls

Single staining in multicolor flow cytometry is essential due to spectral overlap between different fluorophores. Single staining allows you to remove or compensate for this overlap. Spectral overlap should be compensated for every fluorophore used. Remember the fluorophore needs to be the same as the one in your experiment, with a similar brightness and you need to collect enough events to be statistically significant.

4

Viability controls

Dead cells have greater autofluorescence and increased non-specific antibody binding, leading to false positives and reducing the dynamic range. This can make identification of weakly positive samples or rare populations difficult. Forward and side scatter profiles may not be sufficient to remove dead cells. Common viability dyes to identify dead cells include DNA dyes such as Readidrop™ Propidium Iodide and 7AAD or protein binding dyes such as VivaFix™ which are fixable.

5

Fc blocking controls

Fc receptors are found on monocytes, macrophages, dendritic cells and B cells. Antibody binding via Fc receptors can lead to false positives and data that cannot be interpreted. In order to prevent this type of binding, Fc blocking reagents (e.g. Human Fc Seroblock and Murine Fc Seroblock) have been developed which, when added to a staining protocol, can ensure that only antigen specific binding is observed.

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Fluorescence minus one controls

Fluorescence minus one (FMO) controls are important when building multicolor flow cytometry panels as they will help you determine where your gates should be set. When acquiring data, there is fluorescence spread within a detection channel, especially with brighter fluorophores, which will affect other channels. FMO controls are the experimental cells stained with all the fluorophores minus one for each fluorophore in your multicolor panel to detect this influence.

7

Intracellular controls

Intracellular staining requires fixation and permeabilization which can affect antigen detection, autofluorescence, fluorophore brightness and cell morphology, therefore extra controls may be necessary. In addition not all intracellular staining protocols are the same so may require different controls. Isotype controls may not be optimal so additional controls should be considered. These can include controls such as stimulation/blocking experiments and negative/positive controls.

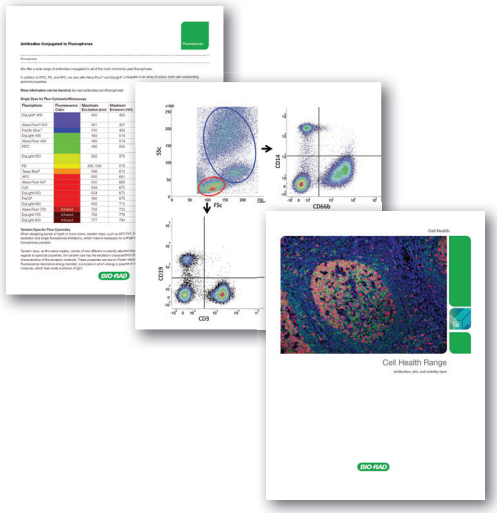
8

Biological controls

Finally in addition to staining and isotype controls, you should also consider biological controls that will enable you to determine staining specificity and experimental limitations. Suitable controls may include negative samples or known positive samples or treated and untreated cells. For some experiments such as cytokine release measurement, an unstimulated and fully stimulated sample is important to determine both positive results and the dynamic range of fluorescence staining. Understanding of your experiment and your sample is important in choosing the right biological control.

Visit **bio-rad-antibodies.com/flowcontrols,** for more information on controls in flow cytometry and practical advice.

Interested to learn more about flow cytometry? We offer a range of application guides, protocols and hands-on tips and tricks. Visit **bio-rad-antibodies/flow-resources** to find out more.



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