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UNMC FLOW CYTOMETRY RESEARCH FACILITY

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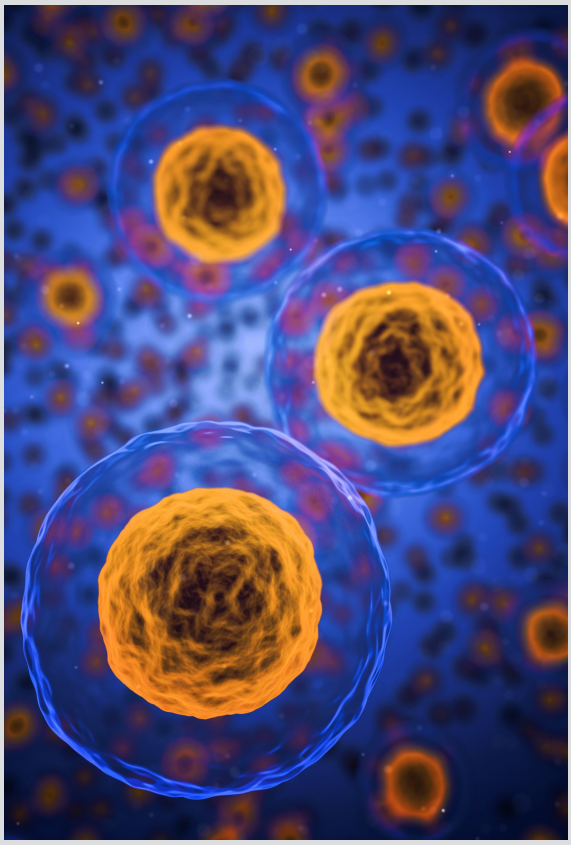
In This Issue



DID YOU KNOW...

Modern Flow Cytometers can analyze tens of thousands of particles per second!

5 BEST PRACTICES FOR ACCURATE FLOW CYTOMETRY RESULTS

Follow best practices to help improve reproducibility and accuracy of your flow data!

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5 BEST PRACTICES FOR ACCURATE FLOW CYTOMETRY RESULTS

AUTOMATED COMPENSATION

- The critical consideration for robust automated compensation is that the controls meet 3 criteria. These have also been called the “3 Rules of Compensation”.
    a. The control must be at least as bright as the experimental sample.
    b. The backgrounds of the positive and negative samples must be identical.
    c. The tube must be acquired at the same voltage and use the exact same fluorochrome as the control.

ISOTYPE CONTROLS

- There is no perfect control for nonspecific binding. Rather, it must be procedurally minimized at
- several levels, including using high-quality antibodies, proper blocking, titration to ensure the
- appropriate concentration of antibody, and the use of proper controls such as FMOs, biological
- controls, internal negative populations, and more.

FLUORESCENCE MINUS ONE (FMO)

- The Fluorescence Minus One, or FMO, is a control that addresses the loss of sensitivity in a given channel.
- The FMO is critical when accurate discrimination is necessary, such as for rare events or dimly expressed markers. During the panel development phase, it is recommended to test all possible FMOs and keep those that are critical to determine the proper gate placement.

OPTIMIZING PMT VOLTAGES

- A good PMT voltage should meet the following criteria:
    - The dimmest cells are in a region where electronic noise (EN) contributes no more than 10-20% of the variance
    - The positive signal is on scale and in the linear region of the detector
- Using the Peak 2 Method, a dim particle is run over a voltage series, and the spread of the data, as measured by CV, is plotted against the voltage, generating a curve. As the voltage increases, CVs decrease until an inflection point is reached. This inflection point represents the point where increasing voltage does not decrease the CV, and is the best starting point for setting voltage.

EXPERIMENT-SPECIFIC QC PROTOCOLS

- This QC is usually performed in the morning, however, instrument status may change over the course of the day.
- Experiment-specific QC can be a very simple addition to the experimental workflow, but provides an invaluable resource in determining how the system is behaving when the experiment is performed and how well the staining process was performed.
- These two controls, using a beadset for instrument performance and a reference control for staining variation, give the researcher an added level of confidence in the performance of instrument and protocol.

Quick Tips - APOPTOSIS

1. Annexin V is a calcium-dependent assay. Without the correct binding buffer containing calcium, there is likely to be little or no Annexin V staining.
2. For optimal results, you must count your cells and titrate your reagents for each cell type you are analyzing.
3. Annexin V binding is not stable and does not fix well, therefore, we recommend your samples be analyzed on a flow cytometer within 1 hour of staining.
4. Single-color controls are critical for gating apoptosis data. Make sure to include a negative control, an Annexin V control, and a PI (or other dead cell stain) control.
5. Make sure to speak with the flow lab about your experiment to ensure the correct instrument and amount of time is booked.

Are You Using The Correct COST-CENTER NUMBER?

Take a moment to double check the billing number you are using for your Flow Cytometry Appointments!

Follow us on Facebook and Twitter for all things #FlowCytometry!

@FlowUNMC

Featured Core User

Name: Yiqian Wang, PhD Student
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Principal Investigator: Ricia Hyde, PhD

My research focuses on identifying and characterizing leukemia stem cells (LSC) in one subtype of acute myeloid leukemia (AML) called Inversion (16) AML (Inv(16)). Flow cytometry helps us to understand the activities of LSCs in inv(16) AML using different types of tests including proliferation and viability, as well as transplantation assays. In addition, we're also able to sort potential LSC subpopulations based on cell surface markers and further test their biological properties in vitro and in vivo.

Need to SORT cells?
Our sorters book up quickly, so make your appointment at least a week before your experiment!

Contact Us

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