Procedures for assaying the association of bacteria or fluorescent latex beads to cells by flow cytometry

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Abstract

Goal:

This document aims to standardize the protocol for assaying the association of bacteria or fluorescent latex beads in eukaryotic cells by flow cytometry.

General considerations:

- There are different models of flow cytometers, so it is necessary to first identify which cytometer suits your experiment best and possibly include protocol adaptations specific to the equipment that will be used.

- Remember to perform the replicates (at least 3 biological replicates) of your experiment on the same cytometer to avoid calibration and reading variations.

Experimental proceedings:

1. Label the bacteria with PKH (see PKH labeling protocol);
2. Infect cell culture with the labeled bacteria in the desired multiplicity of infection (MOI), for example: in Schwann cell culture (ST8814), in a 24-well plate with 40,000 cells per well, we used a MOI of 50:1. In case of fluorescent latex beads, stimulate cells with the desired proportion;
3. Maintain the infection (or stimulation) for the period determined for the experiment, at the appropriate temperature (33˚C for infection with live *leprae*, for example) in 5% CO_2 atmosphere;
4. Discard the supernatant from the culture plate wells, using a pipette, or collect it in properly labeled microtubes for further analysis;
5. Add 150 µL of 5 mM of EDTA into each well for 1 to 2 min at 4 ˚C, giving moderate beats to the side of the plate; * Be careful with the beats on the side of the plate, so as not to spill the contents of the wells of the culture plate and to lose part of the samples.
6. Collect the entire volume contained in each well, washing the well with the solution to be collected, 5 times and transfer the sample to a microtube;
7. Centrifuge the solution at 400 x g for 5 min;
8. Carefully remove the supernatant with the aid of a pipette;
9. Resuspend the pellet in 400 µL of 4% paraformaldehyde solution and keep the sample for 20 min at room temperature. Alternatively (in the case of "fragile" cells) the pellet can be first resuspended in 200 µL of PBS and then in 200 µL of 8% paraformaldehyde;
10. Transfer the solution to the tube (with or without filter) suitable for the cytometer; **The cytometer tube filter is used to prevent clumps of cells, but it may retain some cells. So be

sure to check out the best option for your experiment beforehand.

11. Proceed to the analysis on the cytometer, selecting the population of interest and the fluorescence channel for the used PKH or fluorescent latex beads.
12. To determine the degree of internalization of bacteria, they are labeled with PKH67 (green), and the reading should be made with and without vital dye Trypan Blue (0.4%) in order to distinguish cells containing adhered and/or internalized bacteria. In this assay, termed quenching assay, the Trypan Blue dye is added to "quench" the fluorescence of the extracellular bacteria. The same should be done to evaluate the internalization degree of green fluorescent beads.
13. The percentage of cells with associated bacteria or beads (adhesion + internalization), as well as the percentage of cells containing only internalized bacteria or beads (internalization) is determined comparing the reading with uninfected cultures. The Median fluorescence intensity (MFI) can also be evaluated.

References:

Adapted from: https://www.scbt.com/scbt/pt/resources/protocols/flow-cytometry

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