

Procedures for labeling of bacteria with PKH kit (Fluorescent Cell Linker Kit for General Membrane Labeling - Sigma-Aldrich)

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Abstract

Goal:

This document aims to standardize the protocol for the labeling of bacteria using the Sigma Aldrich kits PKH26 (red) or PKH67 (green).

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General considerations:

- The handling of the material should be done within a Biological Safety Cabin (BSC), according to the bacterial species to be used. For example, for manipulation of *M. leprae*, BSC Class 2 (Cabins for manipulation of pathogenic biological agent, using out-of-laboratory airflow, which provides protection to the material, the manipulator, and the working environment).
- Sterile materials (microtubes, tips and solutions) should be used.
- The kit is stored in a refrigerator.
- The volume of initial bacterial culture to be used in the labeling protocol should correspond to twice the amount of bacteria that will be used in the experiment, because there is loss of bacteria in the processing.

Experimental proceedings:

1. Centrifuge the solution with bacteria (previously quantified for use in the experiment *) at 18,000 x g for 5 minutes to sediment the bacteria;
2. Discard the supernatant, being careful not to lose the pellet;
3. Resuspend the pellet in 1X sterile PBS (same volume as contained in the initial solution);
4. Centrifuge the solution with bacteria at 18,000 x g for 5 minutes;
5. Discard the supernatant;
6. Prepare PKH solution: 1µl of the fluorophore PKH + 99µl of diluent reagent (PKH Kit);
7. Resuspend the bacterial pellet in the 100 µl of the PKH solution.

8. Incubate for 15 minutes at room temperature protecting from the light, vortexing briefly every 5 minutes, being careful not to raise the liquid to the cap and to leak.
9. Add twice the volume of fetal bovine serum (200 ul) - to stop the reaction - and incubate at room temperature for 1 minute;
10. Centrifuge the solution with bacteria at 18,000 x g for 5 minutes;
11. Discard the supernatant and resuspend in the culture medium for the experiment;
12. Quantify the labeled bacteria before use.

References:

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Adapted from:

<https://www.sigmaaldrich.com/catalog/product/sigma/pkh67>

<https://www.sigmaaldrich.com/catalog/product/sigma/pkh26>

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Protocol