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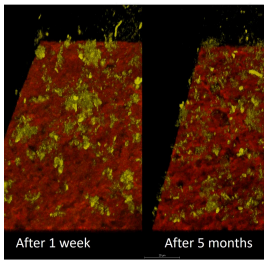
Preservation method for long-term storage of fluorescently labeled cells for microscopy



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Protocol status: Working
We use this protocol and it's working

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Abstract

This method can be used to preserve fluorescently labeled bacterial cells for long-term storage before imaging on a fluorescent microscope. Using this protocol, samples can be saved at 4°C for weeks-months while maintaining strong, easily detectable fluorescence and cell integrity.

Image Attribution

The image was produced by the authors using a Leica SP8 confocal microscope. This is a comparative photo of biofilms expressing a fluorescent protein that were preserved and imaged after 1 week vs 5 months.

Guidelines

This protocol has been successfully used on liquid bacterial cultures and on biofilms on solid plastic material. Cells were imaged using a confocal microscope up to 5 months after preservation. Fluorescence may be maintained longer than 5 months, but has not been tested by the authors.

Materials

Microfuge tubes
Glycerol
Paraformaldehyde
PBS
4ºC storage

Troubleshooting

Safety warnings

Paraformaldehyde is toxic and a skin irritant. Wear appropriate PPE when preparing and working with this solution.

Before start

Prepare the preservation components: 10% glycerol (sterile), 4% paraformaldehyde in PBS pH 7.4 (sterile)

Sample

2m

1 Collect your cells (up to 500 µl) in a sterile microfuge tube. 2m
- This protocol can also be used on solid material containing biofilms.

Preserve

3m

2 To your sample tube, add 250 µL sterile 10% glycerol and 250 µL sterile 4% paraformaldehyde in PBS (pH 7.4). 2m
- If preserving solid material, make sure the entire sample is submerged in the preservation solution.

3 Gently Mix 30s

Storage

1m

4 Store samples at 4 °C until ready to image. 1m