

Ethanol Fixation Protocol for *Sulfolobus acidocaldarius*

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Abstract

This protocol describes the ethanol fixation of *Sulfolobus acidocaldarius*, which is used for immunofluorescence and flow cytometry applications. Ethanol fixation is a widely used method to preserve cell morphology and antigenicity while ensuring long-term sample storage. Alternative fixation methods include methanol and formaldehyde fixation, which offer different advantages depending on the downstream application. This protocol details the step-by-step preparation of ethanol-fixed samples, ensuring optimal preservation for cytometric and imaging analyses. While ethanol fixation is less permanent than e.g., formaldehyde fixation, samples remain stable for several months at 4°C.

Background

Ethanol fixation is commonly used for preserving cellular structures while maintaining compatibility with immunofluorescence and flow cytometry. Compared to formaldehyde fixation, ethanol fixation provides better antigen retention and minimizes autofluorescence, making it a preferred method for flow cytometry applications. This protocol is specifically designed for *Sulfolobus acidocaldarius*, a thermophilic archaeon used in microbiological and cell biological studies. The procedure ensures high-quality sample preservation with minimal processing steps

Materials

Product name	Brand	Manufacturer	Catalogue number	Notes
Bucket with ice	BA	NA	NA	Used to keep samples chilled

Product name	Brand	Manufacturer	Catalogue number	Notes
Labelled 15ml Falcon tubes	Falcon	Thermo Fisher Scientific	NA	One per sample
Absolute ethanol (chilled)	Supelco	Merck	NA	Required for fixation
Bucket with ice	NA	NA	NA	Used to keep samples chilled

Equipment

Equipment name	Brand	Manufacturer	Catalogue number	Notes
Pipettes	Gilson	Gilson		For accurate liquid handling
Refrigerated storage				Required to store fixed samples

Protocol

Step 1: Preparation of Falcon Tubes

1. Label 15ml Falcon tubes, one per sample.
2. Prefill each tube with 1.5 ml of chilled absolute ethanol.
3. Place the tubes on ice and allow to chill

Step 2: Sample Fixation

1. Once ready to sample, add 3 ml of *S. acidocaldarius* culture to the Falcon tube.
2. Invert the tube twice to mix.
3. Place the tube back on ice.

Step 3: Ethanol Addition

1. After 5 minutes, add 1.5 ml of 4°C absolute ethanol to the Falcon tube.
2. Invert twice to mix and return to ice.
3. After another 5 minutes, add 4 ml of 4°C absolute ethanol to reach a final volume of 10 ml (70% ethanol final concentration).

Step 4: Storage

1. Store the samples at 4°C.
2. Samples will remain stable for several months.

Competing interests

The authors declare that they have no conflict of interest.

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