

Leifson Flagella Stain

Learn how to make Leifson flagella stain and use it to stain bacterial flagella.

Materials

Sodium Chloride, 1.5% (item #888880)
 Aqueous Tannic Acid, 3% (item #893000)
 Basic Fuchsin, Alcoholic, 1.2% (item #864170)
 Microscopic Slides (item #292916)
 Glass Bottle (item #716290)

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Item Number
 863193

Procedure

1. To prepare stain solution, mix equal parts sodium chloride solution, tannic acid solution, and basic fuchsin solution and tightly cap.
2. Prepare stain solution as needed and refrigerate.
3. Prepare smears from young cultures on extremely well-cleaned slides.
4. Flood slides with mordant and allow them to stand for 10 minutes in warm weather, or in an incubator in cold weather.
5. Wash with tap water.
6. Dry and examine.
7. Counterstain as needed with methylene blue.

Notes

- In general, the success of flagella staining is contingent on expert practice of the above methods. The difficulty with flagella staining comes from the minuscule diameter (about 0.02–0.03 μm) and the resistance bacteria have to flagella unless the cultures are handled exactly right. The former can be avoided by using a mordant that precipitates on the flagella, thereby increasing the diameter to the visual limit. The latter difficulty can be prevented by handling the cultures such that, upon examination, they are as actively motile as possible, able to swim clear of one another, and allowed to dry on a slide with the smallest amount of damage to the flagella.
- The condition of the slides is imperative to the success of the staining. If possible, use new slides. For previously used slides, clean with dichromate cleaning fluid and wash and rinse in 95% ethyl alcohol. Pass each slide through a flame until the flame turns an orange color. (**Note:** *A high level of experience is required to accurately assess the proper amount of heating necessary.*) Cool slides gradually, in order to re-anneal, and thus minimize breakage.
- If desired, borax methyl blue may be used for 5 to 10 minutes without heat, following the aforementioned staining procedure.
- The above procedure should result in the following:
 - o Flagella–red
 - o Cells–blue (if counterstained)
 - o Capsules (if applicable)–red

Label Information

Flagella Stain, Leifson

Caution: Highly flammable

Date Prepared: _____

Initials of Preparer: _____

Health Risk: 2

Flammability: 3

Reactivity: 2

Applications

The purpose of the solution is to stain bacterial flagella, thereby revealing the presence or absence of flagella as well as their orientation on the cell periphery. These traits can be used to characterize bacteria phenotypically and those that are will possess flagella in various locations extending from the cell membrane.

Reference

American Society for Microbiology Microbe Library. Bacterial flagella stain protocol. <http://www.microbelibrary.org/component/resource/laboratory-test/3153-bacterial-flagella-stain-protocol> (accessed June 6, 2014).

Leifson, Einar. 1930. A method of staining bacterial flagella and capsules together with a study of the origin of flagella. *Journal of Bacteriology* 20 (3):203–11.