DATA SHEET

Product Information

Live Bacterial Gram Stain Kit

Catalog Number: 32000-1 (200 assays)
Components:

Material	Quantity
CF™594 wheat germ agglutinin (WGA), 40X (#32000-1A)	250 ul
DAPI, 125X (#99961)	80 ul

Catalog Number: 32000 (800 assays)
Components:

Material	Quantity
CF™594 wheat germ agglutinin (WGA), (#32000A)	lyophilized solid
DAPI, 125X (#32000B)	320 ul

Technical Information

Storage upon receipt:

- **Store at -20°C in small aliquots**
- **Protect from light**

Excitation/Emission (nm):

- CF™594 wheat germ agglutinin (WGA): 593/614 nm
- DAPI: 358/461 nm, bound to DNA

Storage and Handling:

CF™594 wheat germ agglutinin (WGA) conjugate can be stored at 4°C. For long-term storage, aliquot and freeze at -20°C, protected from light. Under these conditions, the components of the kit is stable for at least one year.

Before use, allow reagents to thaw and centrifuge briefly before opening vials. Tightly reseal all vials before re-freezing. Avoid repeated freezing and thawing.

Caution: DAPI is a nucleic acid binding dye and a known mutagen. Use precaution when handling. Dispose of solution containing DAPI according to your institutional rules and regulations.

Introduction

The Live Bacterial Gram Stain Kit contains two components: CF™594 conjugate of wheat germ agglutinin (WGA) and DAPI solution for distinguishing between gram-negative and gram-positive bacteria. Intact gram-negative bacteria will stain only fluorescent blue with DAPI. Gram-positive bacteria will stain with a fluorescent blue interior, and red fluorescence on the surface of the cells with the CF™594-WGA conjugate. It has been shown that fluorescently labeled wheat germ agglutinin binds specifically to the *N*-acetylglucosamine of the peptidoglycan layer of gram-positive bacteria [1].

This alternative fluorescent gram staining method is designed to distinguish between live gram-negative and gram-positive bacteria without the use of fixatives. Dead cells in a mixed population of gram-positive and gram-negative bacteria may stain variably.

The Live Bacterial Gram Stain Kit was tested on the following bacterial species *Bacillus subtilis* subsp. *subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus epidermidis*. Staining was performed on overnight cultures of these organisms grown in recommended growth media.

Materials

Included in kit:

- **CF™594-wheat germ agglutinin (WGA) conjugate (200 assays kit)**, 40X concentration in 1X PBS with 0.05% sodium azide.
- **CF594™WGA conjugate (800 assays kit)**, lyophilized, dissolve in 1 mL PBS or water to make a 40X stock solution, containing 0.05% sodium azide.
- **DAPI solution**, in water at 125X concentration.

Required materials not included in kit:

- **BSA-NaCl:** 0.25% bovine serum albumin (BSA), 0.15 M NaCl, sterilized by filtration

Protocol

The following protocol is provided only as a guide for researchers. Users should optimize and validate a procedure for their own bacterial samples.

A wash step before staining with CF™594-WGA is not necessary, however, skipping this step may lead to high background. The wash step is to help remove components of the bacterial growth media that may potentially bind to the conjugate. Phosphate buffers such as PBS may not be compatible with CF™594-WGA staining and is not recommended.

Procedure:

1. Harvest bacterial cells by centrifugation at 10,000 x g for 5 minutes in microcentrifuge tubes.
2. Wash cells once in BSA-NaCl buffer by pipetting up and down several times.
3. Pellet cells by centrifugation at 10,000 x g for 5 minutes.
4. Resuspend cells in 50 µl BSA-NaCl.
5. Add CF™594-WGA conjugate to a final concentration of 1X, and mix by pipetting up and down several times. Use a lower concentration (< 1X) if WGA staining pattern does not distinguish between gram-negative and gram-positive bacteria.
6. Incubate cells at room temperature for 10 minutes, protected from light.
7. Pellet cells at 3000 rpm for 5 minutes to remove the WGA staining solution.
8. Resuspend in 50 µl BSA-NaCl.
9. Add DAPI to a final concentration of 1X.
10. Incubate cells at room temperature for 5 minutes, protected from light.
11. Transfer 5 µl of the sample to a slide, apply a glass coverslip, seal, and observe fluorescence on a fluorescence microscope, using appropriate filters.

Notes

- For fluorescence microscopy, it is recommended to view the fluorescence of CF™594-WGA and DAPI using separate band pass optical filters.
- The Live Bacterial Gram Stain Kit is not recommended for use with dead bacterial samples.
- Different bacterial gram-positive species will stain with varied levels of fluorescence intensity. A higher concentration of CF™594-WGA can be used to increase signal intensity; however, this may result in higher background.
- Combining CF™594-WGA and DAPI in a one-step staining procedure can lead to very high background and faint or no specific staining and is not recommended for this kit.
- Staining in 3 M KCl instead of BSA-NaCl may increase fluorescent intensity of CF™594-WGA, but may also lead to some non-specific staining. If this buffer is preferred, it is recommended that users validate this buffer with their organisms.

Reference:

1. Sizemore R.K., Caldwell J.J, and Kendrick A.S. 1990. Appl. Environ. Microbiol. 56(7):2245-2247.