DATA SHEET

Product Information

Bacterial Viability and Gram Stain Kit

Catalog Number: 32001
Components:

Material	Quantity
CF™488A wheat germ agglutinin (WGA), 40X (#32001A)	500 ul
DAPI, 125X (#99961)	80 ul
Ethidium homodimer III (EthD-III), 200X (#32001B)	50 ul

Technical Information

Storage upon receipt:
- **Store at -20°C in small aliquots**
- **Protect from light**

Excitation/Emission (nm):
- CF™488A wheat germ agglutinin (WGA): 490/515 nm
- DAPI: 358/461 nm, with DNA
- Ethidium homodimer III (EthD-III): ~530/~620 nm, with DNA

Storage and Handling:

CF™488A-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 should be 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.

EthD-III stain binds to nucleic acids. The DMSO stock should be handled with extra precaution as DMSO is known to facilitate entry of molecules into tissues.

Dispose of solution containing DAPI and EthD-III according to your institutional rules and regulations.

Table 1. Staining pattern using Bacterial Viability and Gram Stain Kit

	Gram-positive bacteria	Gram-negative bacteria
Live cells	Blue interior Green surface	Blue
Dead cells	Red interior Green surface	Red

Introduction

The Bacterial Viability and Gram Stain Kit contains three components: CF™488A-WGA, DAPI, and EthD-III solutions for distinguishing between gram-negative and gram-positive, as well as live versus dead bacteria. 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]. EthD-III is a nucleic acid binding dye that is cell impermeable and will stain cells with compromised cell membranes. On the other hand, cells with intact membranes will stain fluorescently blue with DAPI. The staining pattern is summarized in Table 1.

This kit does not require the use of fixatives. The Bacterial Viability and 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.

If samples are prepared as recommended in the protocol below, this kit contains enough material for 200 assays.

Materials

Included in kit:

- **CF™488A wheat germ agglutinin (WGA) conjugate**, 40X in 1X PBS with 0.05% sodium azide.
- **DAPI solution**, 125X in water.
- **Ethidium homodimer III (EthD-III) solution**, 200X in DMSO.

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™488A-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™488A-WGA staining and is not recommended.

The bacterial viability component of this assay is based on the integrity of the cell membrane. The ability of this kit to determine other techniques for discriminating bacterial viability should be evaluated and compared to a reference procedure before applying the use of this kit.

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™488A-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 and EthD-III to a final concentration of 1X each.
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™488A-WGA, DAPI, and EthD-III using separate bandpass optical filters.
- Different bacterial gram-positive species will stain with varied levels of fluorescence intensity. A higher concentration of CF™488A-WGA can be used to increase signal intensity; however, this may result in higher background.
- Combining CF™488A-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™488A-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.