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- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Revised: July 29, 2020

Product Information

Bacterial Viability and Gram Stain Kit

Catalog Number: 32001

Kit Components

Component	Size
32001A: CF®488A Wheat Germ Agglutinin (WGA), 40X	250 uL
32001B: Ethidium Homodimer III (EthD-III), 200X in DMSO	50 uL
99961: DAPI, 125X	80 uL

If samples are stained according to the protocol below, the kit contains enough material for 200 assays.

Storage and Handling

DAPI and EthD-III can be stored at 4°C or -20°C. Store CF®488A WGA conjugate in aliquots at -20°C, protected from light. Avoid repeated freeze-thaw cycles. The components are stable for at least one year from date of receipt if stored as recommended.

Spectral Properties

DAPI Ex/Em: 358/461 nm (with DNA)
CF®488A Ex/Em: 490/515 nm
Ethidium homodimer III (EthD-III) Ex/Em: 532/625 nm* (with DNA)
*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

Product Description

The Bacerial Viability and Gram Stain Kit provides a convenient assay for distinguishing between gram-negative and gram-positive (green), as well as live versus dead (red) bacteria, by fluorescence microscopy. All of the bacteria will be stained with DAPI (blue) as a counterstain.

Fluorescently labeled wheat germ agglutinin (WGA) binds specifically to the N-acetylglucosamine of the peptidoglycan layer of gram-positive bacteria, functioning as a fluorescent Gram stain. Ethidium Homodimer III (EthD-III) is a nucleic acid binding dye that is membrane-impermeant, which selectively stains cells with compromised plasma membranes. All cells stain blue with the membrane-permeant DNA dye DAPI. The expected staining pattern of the different types of bacteria is summarized in Table 1.

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.

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 and blue interior Green surface	Red and blue

Assay Protocol

1. Harvest bacterial cells by centrifugation at 10,000 x g for 5 minutes in microcentrifuge tubes and remove the supernatant.
2. Optional: Wash cells once in 150 mM NaCl by gently pipetting up and down.
3. Pellet cells by centrifugation at 10,000 x g for 5 minutes and remove the supernatant.
4. Resuspend cells in 50 uL of 150 mM NaCl.
5. Add CF®488A WGA conjugate to a final concentration of 1X (ie, add 1.25 uL of WGA to 50 uL sample), and mix by pipetting up and down several times.

   Note: Different gram-positive bacteria species will stain with different levels of fluorescence intensity. The concentration of WGA may require optimization to distinguish between gram-negative and gram-positive bacteria.
6. Incubate cells at room temperature for 10 minutes, protected from light.
7. Pellet cells by centrifugation at 10,000 x g for 5 minutes and remove the WGA staining solution.
8. Resuspend in 50 uL of 150 mM NaCl.
9. Add DAPI and EthD-III to a final concentration of 1X each (ie, add 0.25 uL EthD-III and 0.4 uL DAPI to 50 uL sample), and mix by pipetting up and down several times.

   Note: Combining CF®488A WGA and DAPI in a one-step staining procedure can lead to very high background and low signal and is not recommended.
10. Incubate cells at room temperature for 5 minutes, protected from light.
11. Transfer 5 uL of the sample to a slide, apply a glass coverslip, and seal with CoverGrip™ Coverslip Sealant (23005) or nail polish.
12. Image the labeled cells by fluorescence microscopy. CF®488A WGA can be imaged using a FITC filter set, EthD-III can be imaged using Texas Red® or Cy®3 filter sets, and DAPI can be imaged using a DAPI filter set.

Related Products

Catalog number	Product
30027	Viability/Cytotoxicity Assay for Bacteria Live & Dead Cells
32000	Live Bacteria Gram Stain Kit
40101	BactoView Live™ Red
40102	BactoView Live™ Green
40069	PMAxx™ Dye for viability PCR, 20 mM in water
40013	PMA Dye for viability PCR
40019	PMA Dye for viability PCR, 20 mM in water
E90002	PMA-Lite™ LED Photolysis Device
31033-31037; 31050, 31051, 31053	Real-Time PCR Bacterial Viability Kits (choose from kits for 8 bacterial strains)
32002-32013	Live-or-Dye™ Fixable Viability Staining Kits
29021-29029; 29059; 29064	CF® Dye Wheat Germ Agglutinin (WGA)
70020	SynaptoGreen™ C4 membrane stain
70021	SynaptoRed™ C2 membrane stain
10063	CTC, bacterial respiration dye
31062	Yeast Vitality Staining Kit
31063	Yeast Viability Staining Kit
31064	Yeast Fixable Live/Dead Staining Kit
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells

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