Revised: September 28, 2012

Product Information

Live Bacterial Gram Stain Kit

Catalog numbers:
32000-1 (200 assays)
32000 (800 assays)

Components:

Component	32000-1	32000
CF™594 wheat germ agglutinin (WGA)	250 uL (40X stock solution) (#32000-1A)	1 vial lyophilized solid (#32000A) Reconstitute in 1 mL PBS or dH_2O before use to obtain a 40X stock solution.
DAPI, 125X	80 uL (#99961)	320 uL (#32000B)

Spectral Properties:

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

Storage and Handling:

DAPI can be stored at 4ºC. Store CF™594-WGA lyophilized solid at -20°C, protected from light. Store CF™594 conjugate stock solution 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.

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

Product Description

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 with blue fluorescent DAPI only. Gram-positive bacteria will stain with CF™594-WGA conjugate and DAPI, resulting in blue interior and red surface staining. 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].

The Live Bacterial Gram Stain Kit is not recommended for use with dead bacterial samples. Dead cells in a mixed population of gram-positive and gram-negative bacteria may stain variably. The Bacterial Viability and Gram Stain Kit (catalog #32001) is designed for gram staining and distinguishing live and dead bacteria.

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.

Reference:

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

Protocol

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

Materials required but not provided:

BSA-NaCl: 0.25% bovine serum albumin (BSA), 0.15 M NaCl, sterilized by 0.2 um filtration.
Note: 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 strains.

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.
   Note: This wash step is optional. It removes components of the bacterial growth media that may potentially bind to the conjugate and increase background staining.
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.
   Note: Different bacterial gram-positive 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 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 each.
   Note: Combining CF™594 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 µl of the sample to a slide, apply a glass coverslip, seal, and observe fluorescence on a fluorescence microscope, using appropriate filters.
    Note: For fluorescence microscopy, it is recommended to view the fluorescence of CF™594-WGA and DAPI using separate bandpass optical filters.

Catalog #	Product Name	Unit Size
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells	100-1000 assays
32001	Bacterial Viability and Gram Stain Kit	200 assays
40013	PMA™ (propidium monoazide)	1 mg
40019	PMA™ (propidium monoazide), 20 mM in water	100 uL

Please visit www.biotium.com to view our full selection of products featuring bright and photostable CF™ dyes, as well as a wide selection of innovative products for life science research.