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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- 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

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Product Information

BactoSpore™ Bacterial Stains

Product List

Cat. No.	Unit Size	Product Name	Abs/Em	Detection Channel
40119-T	20 uL	BactoSpore™ 485/500 Membrane Stain, 500X in EtOH	484/504 nm	FITC
40119	100 uL			
40120-T	20 uL	BactoSpore™ 488/540 Nucleic Acid Stain, 500X in DMSO	488/536 nm (with DNA)	FITC*
40120	100 uL			

*BactoSpore™ 488/540 Nucleic Acid Stain also is detectable in the PE flow cytometry channel off the blue 488 nm laser.

Storage and Handling

Store at 4°C and protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

BactoSpore™ 488/540 is a potentially harmful DNA binding dye with unknown toxicity. Exercise universal laboratory safety precautions when handling the dye.

Product Description

Bacterial endospores are tough dormant structures formed by certain strains of bacteria, including *Bacillus* and *Clostridium* species, in response to nutrient deprivation and other stressors. Endospore formation allows these bacteria to survive in a non-replicative state until growth conditions improve, at which point the spores can germinate to allow vegetative cell replication. Endospores provide a reservoir of potentially infectious bacteria that are resistant to disinfectants, heat, and other decontamination treatments. In addition, the spore coat is highly impermeant and resistant to staining with bacterial detection reagents, which poses a challenge for studying endospore formation and inactivation.

BactoSpore™ Stains are fluorescent dyes optimized at Biotium for staining endospores. The dyes also stain both live and dead bacteria of gram-positive and gram-negative strains. In *B. subtilis*, the dyes stain both vegetative cells and endospores and have been validated for detection by fluorescence microscopy and flow cytometry.

BactoSpore™ 485/500 Membrane Stain is a green fluorescent lipophilic membrane dye for the FITC channel. BactoSpore™ 488/540 Nucleic Acid Stain is a yellow fluorescent nucleic acid dye that is detectable in both the FITC channel and the PE channel for flow cytometry.

See Related Products for BactoView™ Dead Stains and BactoView™ Viability Kits for live/dead bacterial discrimination.

Considerations for Staining with BactoSpore™ Bacterial Stains

- BactoSpore™ Stains cannot be used to distinguish bacteria from eukaryotic cells, because they will stain other cell types as well.
- When combining BactoSpore™ Stains with other stains we recommend titrating the concentration of each stain separately and together to find the optimal concentration and staining protocol (for example, co-incubation of the stains vs. sequential staining).
- BactoSpore™ staining of vegetative cells can withstand fixation with formaldehyde after staining.

Experimental Protocols

Bacteria and endospore staining protocol

This protocol has been developed for staining laboratory bacteria strains in liquid culture. Optimization may be needed for other sample types.

1. Grow your cells in the appropriate growth medium and growth conditions. We typically grow bacteria overnight at 37°C.

   Note: Endospore induction may take up to seven days. See below for an example *B. subtilis* sporulation protocol.

2. Add BactoSpore™ Stain to the bacterial sample at a final concentration of 1X. For example, if the sample volume is 500 uL, add 1 uL of BactoSpore™ Stain and mix well.

   Notes:
   a. You may stain samples in medium or in PBS or similar buffer.

   b. For smaller sample volumes, you may prepare an intermediate dilution of the stain. For example, add 1 uL of BactoSpore™ Dead Stain to 9 uL of buffer, mix well, and then add 1 uL of this intermediate dilution to 50 uL of sample.

   c. Dye concentration may be optimized for different cell or sample types.

3. Incubate at room temperature for 30 minutes, in the dark.

4. Stained cells can be analyzed directly by microscopy or flow cytometry in the FITC channel.

   Tips:
   a. Washing is optional but may be performed to reduce background if needed. To wash, collect the stained cells or spores by centrifugation at 15,000-20,000 xg for 5 minutes. Discard the supernatant and resuspend in fresh buffer or medium (without stain added).

   b. For fluorescence microscopy, you may mount 5 uL of the sample on a slide with an 18 mm coverslip. Alternatively, you may pipet 100 uL of sample into a 96-well optical bottom plate. Image cells in the appropriate detection channel (see Product List).

   Note: Coating the slide or wells with Cell-Tak™ adhesive to immobilize the cells or spores can facilitate imaging of bacteria by microscopy.

   c. For flow cytometry, dilute the sample in FACS wash buffer (PBS + 1% serum) or similar buffer. You may need to dilute the sample 10-fold or more to achieve the desired flow rate. Detect cells or spores in the appropriate detection channel (see Product List).

   d. BactoSpore™ 488/540 Nucleic Acid Stain is detectable in both the FITC and PE channel with 488 nm blue laser excitation, and may also show fluorescence in the PerCP channel.

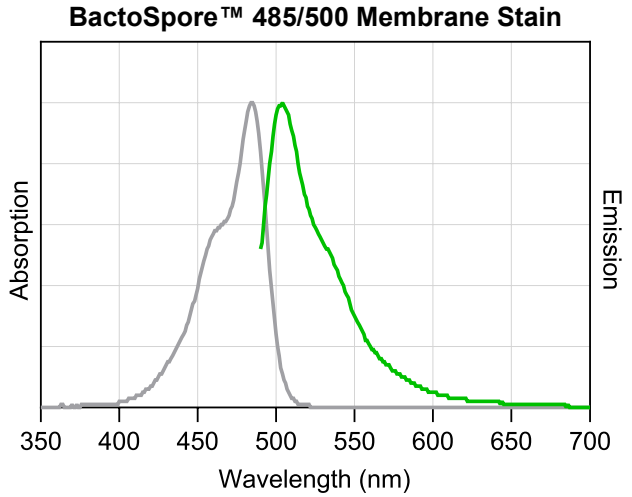

Figure 1. BactoSpore™ 485/500 Membrane Stain absorbance and emission spectra (in methanol).

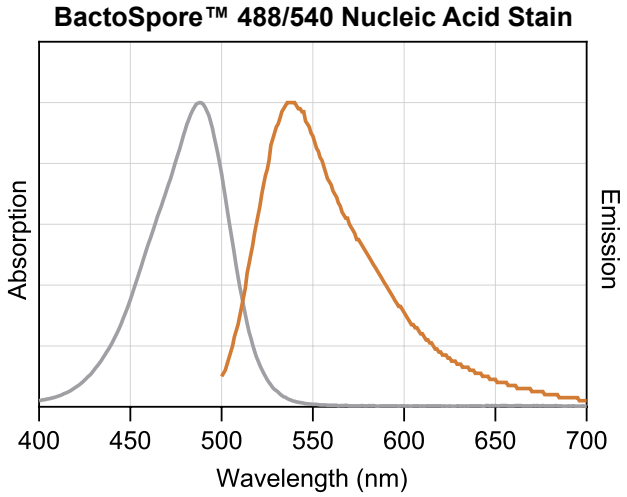

Figure 2. BactoSpore™ 488/540 Nucleic Acid stain absorbance spectra (in methanol) and emission spectra (with DNA in TE buffer).

***B. subtilis* sporulation protocol**

The following is an example of a protocol that we have used to generate *B. subtilis* endospore samples for staining with BactoSpore™ Stains. Other protocols may be optimal for your experimental system or other endospore-forming bacterial strains.

Note: Materials listed below are not provided.

1. Prepare Difco™ Sporulation Medium (DSM):
   a. Combine the following by stirring until fully dissolved:

Component	Amount
Bacto nutrient broth (Difco™)	8 g
10% (w/v) KCl in water	10 mL
1.2% (w/v) $MgSO_4 \cdot 7H_2O$ in water	10 mL
dH_2O	~900 mL

   b. Adjust pH to 7.6 with NaOH.
   c. Add dH_2O to bring the total volume to 1 L total.
   d. Autoclave to sterilize and allow to cool.
   e. Add the following filter-sterilized solutions to 1 L of autoclaved solution from step 1d:

Component	Amount
1 M $Ca(NO_3)_2$ in water (sterile)	1 mL
0.01 M $MnCl_2$ in water (sterile)	1 mL

2. Day 0: Inoculate a 3 mL culture of *B. subtilis* in complete growth medium (e.g., LB or TSB). Grow at 37°C overnight with shaking.
3. Days 1-7: Inoculate a culture of *B. subtilis* in DSM and induce sporulation.
   a. Measure OD600 of the overnight culture from step 2.
   b. Centrifuge 1 mL of cells and resuspend in 1 mL of DSM.
   c. Inoculate 10 mL of DSM with *B. subtilis* at OD600=0.1.
   d. Incubate the DSM culture at 37°C with shaking for 7 days to induce sporulation.

      Note: We have found that spore samples may be stored for later staining at 4°C for up to three weeks provided the culture medium remains uncontaminated. However, different storage conditions may be optimal if you wish to germinate spores.
4. Optional: You may enrich the culture for endospores by using heat, lysozyme, and detergent to lyse and remove residual vegetative cells and endospore mother cell debris.
   a. Transfer 1 mL of DSM culture to a microcentrifuge tube and heat at 60°C for 1 hour.
   b. Centrifuge the culture for 10 minutes at 10,000 xg at 4°C to pellet the endospores. Discard the supernatant.
   c. Suspend the pellet in 1 mL of 50 mM Tris-HCL pH 6.9 containing 50 ug/mL chicken egg white lysozyme. Incubate for 1 hour at 37°C.
   d. Centrifuge the sample to pellet the endospores as in step 4b and discard the supernatant.
   e. Resuspend the pellet in 1 mL of dH2O to wash. Centrifuge as in step 4b and discard the supernatant.
   f. Resuspend the pellet in 1 mL of 0.05% SDS in dH2O by vortexing. Centrifuge as in step 4b and discard the supernatant.
   g. Wash the pellet three times with dH2O as in step e. Resuspend the enriched endospore pellet in dH2O.

Related Products

Cat. No.	Product
40107-40113	BactoView™ Dead Stains
32019-32020	BactoView™ Viability Kits
40101-40102	BactoView™ Live Fluorescent Bacterial Stains
40069	PMAxx™ Dye, 20 mM in H_2O
40013, 40019	PMA (Propidium Monoazide)
E90006	PMA-Lite™ 2.0 LED Photolysis Device
31033, 31033-X	PMA Real-Time PCR Bacterial Viability Kit, *Salmonella enterica* (invA)
31034	PMA Real-Time PCR Bacterial Viability Kit, *Mycobacterium tuberculosis* (groEL2)
31035	PMA Real-Time PCR Bacterial Viability Kit, *Staphylococcus aureus* (nuc)
31036	PMA Real-Time PCR Bacterial Viability Kit, *Staphylococcus aureus* (mecA)
31037, 31037-X	PMA Real-Time PCR Bacterial Viability Kit, *E.coli O157:H7* (Z3276)
31050, 31050-X	PMA Real-Time PCR Bacterial Viability Kit, *E.coli* (uidA)
31051, 31051-X	PMA Real-Time PCR Bacterial Viability Kit, *Listeria monocytogenes* (hly)
31053	PMA Real-Time PCR Bacterial Viability Kit, *Legionella pneumophila* (mip)
10063	CTC (5-Cyano-2,3-ditolyl tetrazolium chloride)
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells
32000	Live Bacterial Gram Stain Kit
31062	Yeast Vitality Staining Kit
31063-1, 31063-2, 31063-3	Yeast Viability Staining Kit
31064	Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit

Please visit our website at www.biotium.com for information on our other products for microbiology, including bacterial and yeast viability stains, bacterial gram stains, yeast organelle stains, viability PCR reagents, and more.

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