

-Bacstain- Bacterial Viability Detection Kit – DAPI/PI

Technical Manual

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/bs08.pdf>

General Information

-Bacstain- Bacterial Viability Detection Kits are series of products for fluorescent double-staining of bacteria. By combining different types of fluorescent stain, staining images can be acquired for each index (membrane impairment, respiratory activity, and esterase activity). Bacterial viability is generally assessed by colony formation in nutrient-agar medium. However, this requires a long culturing time, and it is hard to recognize the growth of viable but nonculturable (VNC) bacteria.

However, fluorescent staining does not require bacterial culture, and enables viability assessment by rapid and simple protocols.

-Bacstain- Bacterial Viability Detection Kit – DAPI/PI uses bacterial membrane damage as an index. DAPI is a minor groove binder specific to the AT sequence of DNA and permeates into bacteria to stain nucleic acids regardless of membrane damage. PI is a parallel intercalator into the DNA double helix that stains nucleic acid; it passes only through damaged bacterial membranes. Thus, this kit can be used to measure the ratio of membrane-damaged bacteria to total bacteria on analysis of the fluorescent images from each stain.

Code	Product name	Combination
BS08	-Bacstain- Bacterial Viability Detction Kit - DAPI/PI	Nucleic acids/Membrane impairment
BS09	-Bacstain- Bacterial Viability Detction Kit - CTC/DAPI	Respiratory activity/Nucleic acids
BS10	-Bacstain- Bacterial Viability Detction Kit - CFDA/PI	Esterase activity/Membrane impairment

Kit Contents

DAPI Solution 25 µl × 4
PI Solution 25 µl × 4

Conditions

Store at ≤-20°C

Required Equipment and Materials

- Incubator
- Micropipettes (20 µl and 1,000 µl)
- PBS(-) or saline
- Fluorescence microscope

Precaution

Centrifuge the tube briefly before opening the cap because the contents may be on the tube wall or cap.

General Protocol

Each reagent included in this kit is sufficient for 100 samples.

- 1) Equilibrate the DAPI and PI Solutions at room temperature by leaving the tubes for 30 min in the dark.
NOTE: Since PI is a potential mutagen, handling and disposal of this reagent should be considered with care.
- 2) Adjust the bacterial suspension to 10^8 – 10^9 bacteria/ml with PBS(-) or saline.
- 3) Transfer 1 ml of the bacterial suspension to a microtube.
- 4) Centrifuge the suspension (5,000 x g, 5 min).
- 5) Remove the supernatant, add 1 ml of PBS(-) or saline, and resuspend the bacteria.
- 6) Repeat steps 4) and 5) twice.
- 7) Add 1 µl of DAPI Solution to the 1-ml bacterial suspension and vortex to mix well (final concentration of DAPI: 2.8 µmol/l).
- 8) Incubate the bacterial suspension at room temperature for 5 min.
- 9) Add 1 µl of PI Solution to the 1-ml bacterial suspension and vortex to mix well (final concentration of PI: 1.4 µmol/l).
- 10) Incubate the bacterial suspension at room temperature for 5 min.
- 11) Observe the bacteria under a fluorescence microscope.

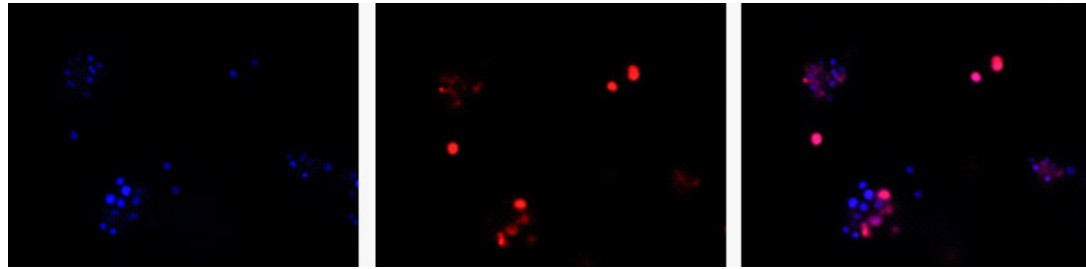
Component name	Maximum Ex/Em (nm)	Recommended filter	Excitation	Emission
DAPI Solution	360/460	DAPI	320–400 nm	410–510 nm
PI Solution	530/620	TRITC	520–570 nm	535–675 nm

**Experimental
example-1**

Double-staining of *Staphylococcus aureus* (a Gram-positive bacterium)

- 1) Two test-tubes containing *Staphylococcus aureus* in 5 ml of liquid medium (TSBYE) were prepared and cultured at 37°C for 14–16 h. One tube was used as a live bacterial sample, and the other was autoclaved at 121°C for 30 min to prepare a sterilized sample.
- 2) The samples prepared in step 1 were diluted with PBS(-) to adjust the bacterial suspension to 10^8 – 10^9 bacteria/ml, then the live and sterilized samples were mixed at a ratio of 7:3.
- 3) One milliliter of bacterial suspension was transferred to a microtube.
- 4) The bacterial suspension was centrifuged at 5,000 x g for 5 min.
- 5) The supernatant was removed and 1 ml of PBS(-) was added to the tube.
- 6) Steps 4) and 5) were repeated twice.
- 7) One microliter of DAPI Solution was added to the bacterial suspension and mixed well.
- 8) The bacterial suspension was incubated at room temperature for 5 min.
- 9) One microliter of PI Solution was added to the bacterial suspension and mixed well.
- 10) The bacterial suspension was incubated at room temperature for 5 min.
- 11) The bacteria were observed under an epi-illumination fluorescence microscope.

All bacteria are stained by DAPI(blue), bacteria with injured cell membrane are stained by PI(red).



DAPI

Excitation filter 320–400 nm
Emission filter 410–510 nm

PI

Excitation filter 520–570 nm
Emission filter 535–675 nm

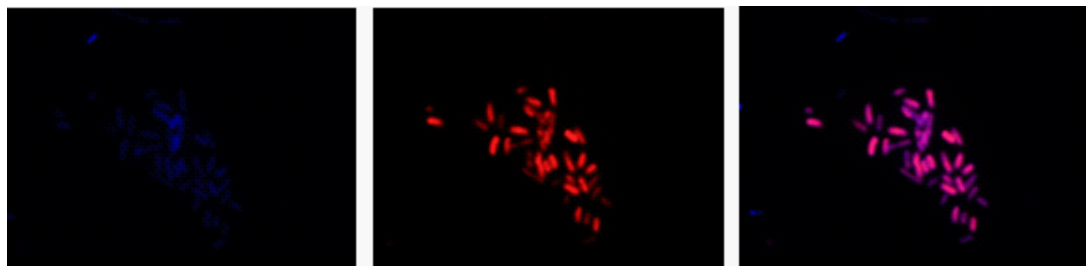
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**Experimental
example-2**

Double-staining of *Escherichia coli* (a Gram-negative bacterium)

- 1) Two test-tubes containing *Escherichia coli* in 5 ml of liquid medium (TSBYE) were prepared and cultured at 37°C for 14–16 h. One tube was used as a live bacterial sample, and the other was autoclaved at 121°C for 30 min to prepare a sterilized sample.
- 2) The samples prepared in step 1 were diluted with PBS(-) to adjust the bacterial suspension to 10^8 – 10^9 bacteria/ml, then the live and sterilized samples were mixed at a ratio of 1:1.
- 3) One milliliter of bacterial suspension was transferred to a microtube.
- 4) The bacterial suspension was centrifuged at 5,000 x g for 5 min.
- 5) The supernatant was removed and 1 ml of PBS(-) was added to the tube.
- 6) Steps 4) and 5) were repeated twice.
- 7) One microliter of DAPI Solution was added to the bacterial suspension and mixed well.
- 8) The bacterial suspension was incubated at room temperature for 5 min.
- 9) One microliter of PI Solution was added to the bacterial suspension and mixed well.
- 10) The bacterial suspension was incubated at room temperature for 5 min.
- 11) The bacteria were observed under an epi-illumination fluorescence microscope.

All bacteria are stained by DAPI(blue), bacteria with injured cell membrane are stained by PI(red).



DAPI

Excitation filter 320–400 nm
Emission filter 410–510 nm

PI

Excitation filter 520–570 nm
Emission filter 535–675 nm

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If you need more information, please contact Dojindo technical service.

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