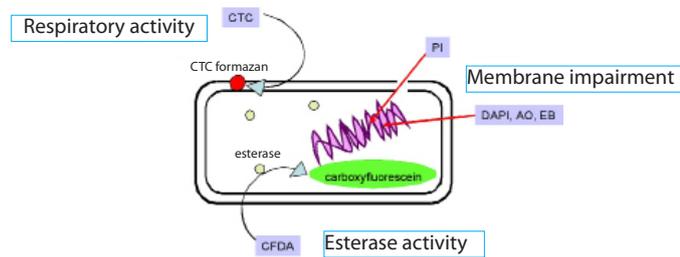


Introduction

-Bacstain- series offer several kinds of bacterial fluorescence staining dye and can be applied for microbial cell viability assay in different principles.

DAPI is an AT-sequence specific DNA intercalator that attaches to DNA at the minor groove of the double helix. -Bacstain- DAPI solution is a Ready-to-Use solution.



Kit contents

DAPI aqueous solution (25 μ l \times 4, 1 mg/ml)

Storage

Store at 0-5°C.

Required Equipment

- Fluorescence microscope (ultraviolet excitation light, blue emission filter)
- or Flow cytometer (ultraviolet light, blue emission filter)
- Micropipette (20 μ l, 1,000 μ l)

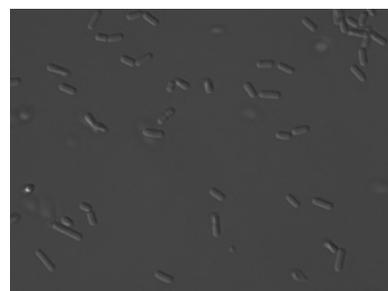
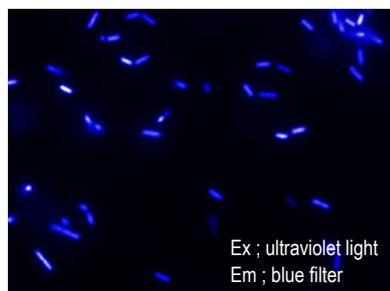
Precaution

- This kit includes microtubes containing solutions. Since there is a possibility that the droplets might attach to the inside walls or caps, please shake them down prior to open.

Staining Procedure

- 1) Allow DAPI solution to stand at room temperature for 30 minutes. Solution should be protected from light ^{a)}.
- 2) Resuspend the organisms with PBS(-) or saline and adjust the number of cells to 10⁶ cells/ml (flow cytometry) or 10⁸-10⁹ cells/ml (microscopy).
- 3) Add 1 μ l of DAPI solution into the 1 ml of microbial cell suspension and vortex gently to mix.
- 4) Incubate the microbial cells at room temperature for 5 minutes.
- 5) Analyze the stained cells by flow cytometer or under a microscope.

a) Since DAPI may be carcinogenic, please be careful in its handling/disposing.



Left: Fluorescence image
Right: Bright field image

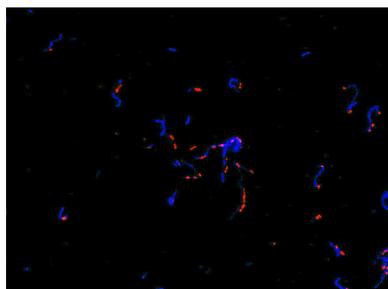
DAPI-stained *B. cereus*

Number of Tests Possible

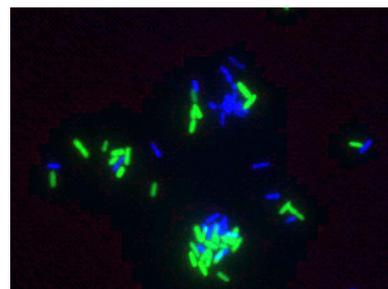
This kit can perform at least 100 tests for the flow cytometric assay and microscopic assay by following the protocol described herein.

Doublestaining (Optional)

If -Bacstain- CTC Rapid Staining Kit or -Bacstain- CFDA solution is used with DAPI, double-staining examination can be performed.



L. casei were stained with CTC, and then counterstained with DAPI.



B. cereus were stained with CFDA, and then counterstained with DAPI.

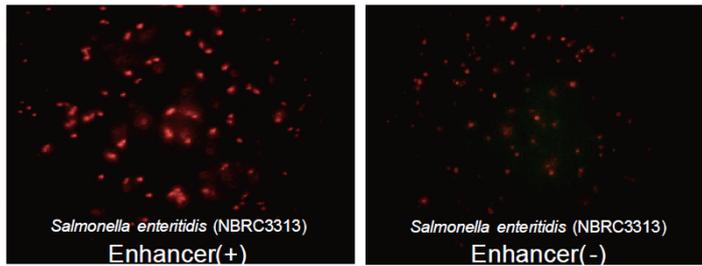
Relevant Products

-Bacstain- CTC Rapid Staining Kit (for Flow cytometry)

-Bacstain- CTC Rapid Staining Kit (for Microscopy)

CTC has been used by many researchers to evaluate the microbial respiratory activity.

-Bacstain- CTC Rapid Staining Kit allows quick and high-sensitivity CTC-staining.



Right-image : without Enhancing reagent
Left-image : with Enhancing reagent

Ex filter : Blue
Em filter : Red

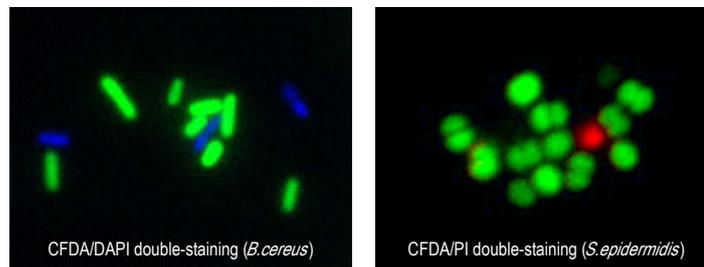
CTC staining efficiencies were compared in with or without enhancing reagent condition.

-Bacstain- CFDA solution

CFDA is widely used as an indicator for the measurement of esterase activity.

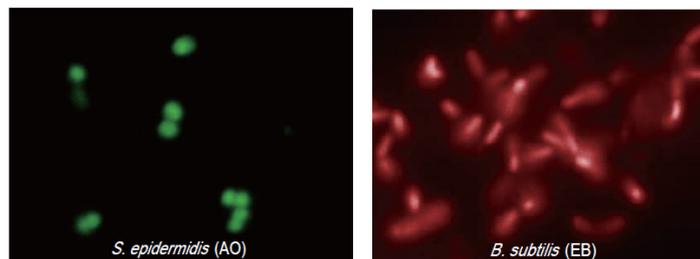
-Bacstain- CFDA solution is provided as Ready-to-Use DMSO solution.

Fluorescent carboxyfluorescein is produced from non fluorescent CFDA by the esterase in the microbial cell.



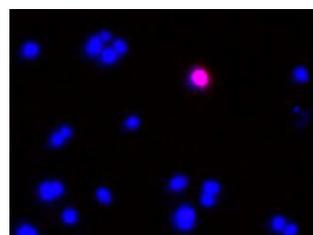
-Bacstain- AO solution, EB solution

AO and EB are nucleic acid staining dyes and can be applied for bacteria either with membrane impairment or with membrane intact



-Bacstain- PI solution

PI is a nucleic acid staining dye. Membrane-injured cells are stained with red emission by PI.



Double-staining of *S.epidermidis* (DAPI/PI)
Red fluorescence represents membrane-injured cells

Products	Code	Maximum Ex/Em(nm)	Number of assays
CTC Rapid Staining Kit (for Flow cytometry)	BS01	430, 480/630	100
CTC Rapid Staining Kit (for Microscopy)	BS02	430, 480/630	100
CFDA solution	BS03	493/515	100
DAPI solution	BS04	360/460	100
AO solution	BS05	420-460/630-650(ssDNA)	100
		500/520(dsDNA)	
EB solution	BS06	520-525/615	100
PI solution	BS07	530/620	100

These products were developed by joint-research with Fukuoka Industrial Technical Center in Japan.

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

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