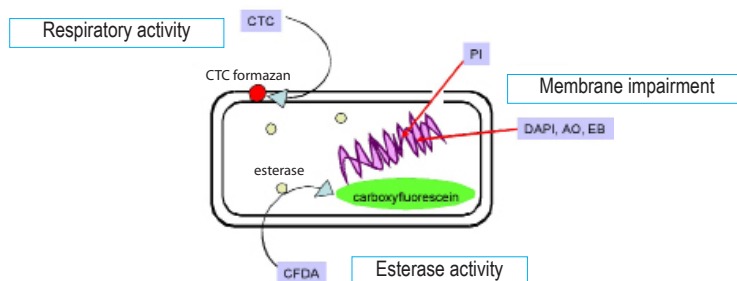


### Introduction

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

Propidium iodide (PI) is an ethidium bromide analog that emits red fluorescence upon intercalation with double-stranded DNA. Though PI does not permeate viable cell membranes, it passes through injured cell membranes and stains the nuclei. PI is often used in combination with a fluorescein compound, such as CFDA, for simultaneous staining of viability and membrane injury.



### Kit contents

PI aqueous solution (25  $\mu$ l $\times$ 4, 1 mg/ml)

### Storage

Store at  $\leq -20^{\circ}\text{C}$

### Required Equipment

- Fluorescence microscope (blue or green excitation light, red emission filter) or Flow cytometer (488 nm or 533 nm laser, red emission filter)
- Micropipette (20  $\mu$ l, 1,000  $\mu$ 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 PI solution to stand at room temperature for 30 minutes for thawing. Solution should be protected from light <sup>a)</sup>.
- 2) Resuspend the organisms with PBS(-) or saline and adjust the number of cells to  $10^8$  cells/ml (flow cytometry) or  $10^8$ - $10^9$  cells/ml (microscopy).
- 3) Add 1  $\mu$ l of PI 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.

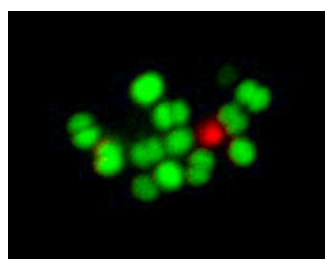
a) Since PI may be carcinogenic, please be careful in its handling/disposing.

### Number of Tests possible

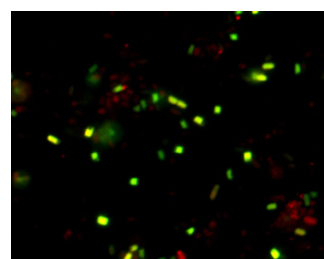
This kit can perform at least 100 tests for the flow cytometric assay and the microscopic assay by following the protocol described herein.

### Doublestaining (Optional)

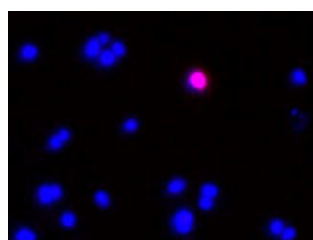
-Bacstain- CFDA solution and -Bacstain- DAPI solution can be applied for double-staining examination with -Bacstain- PI solution.



Double-staining of *S.epidermidis*  
(CFDA / PI)



Double-staining of *E.coli*  
(CFDA / PI)



Double-staining of *S.epidermidis*  
(DAPI / PI)

### References

- 1) N. Yamaguchi and M. Nasu, " Flow cytometric analysis of bacterial respiratory enzymatic activity in the natural aquatic environment ", *J. Appl. Microbiol.*, **1997**, *83*, 43.

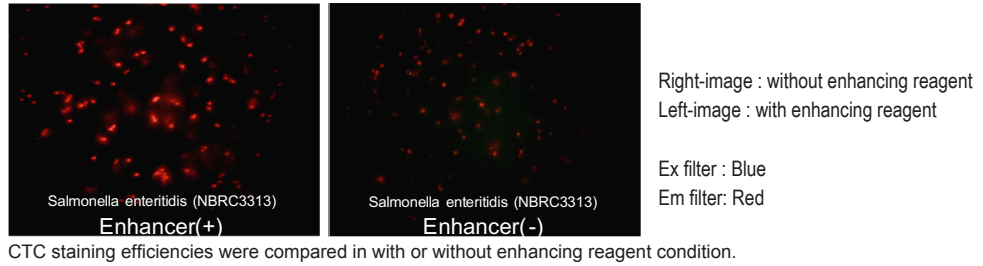
**Relevant products**

**-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.

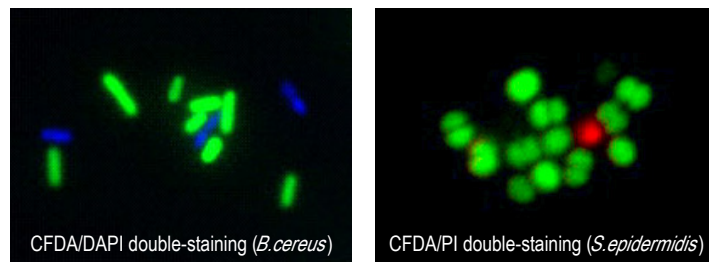


**-Bacstain- CFDA solution**

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

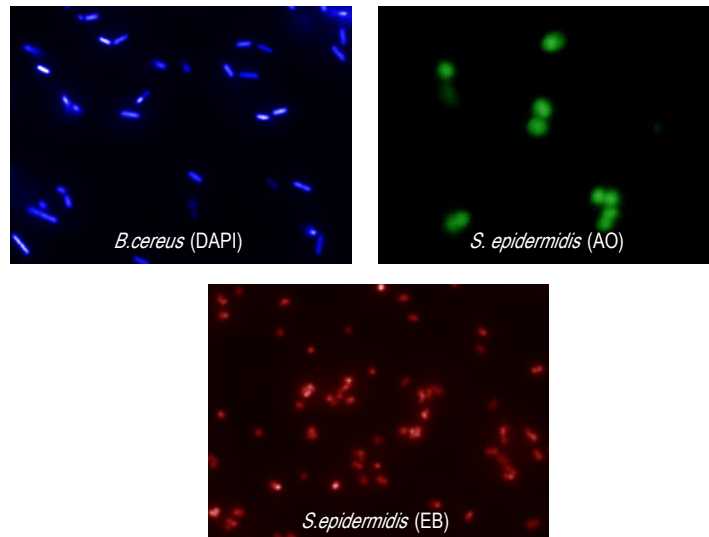
-Bacstain- CFDA solution is provided as Ready-to-Use DMSO solution.

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



**-Bacstain- DAPI solution , AO solution, EB solution**

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



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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BS07: -Bacstain- PI solution