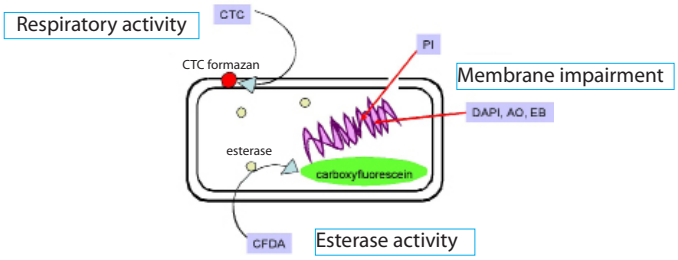
-*Bacstain*- CFDA solution

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/bs03.pdf

Introduction

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



Kit contents

CFDA DMSO solution (375 µl×4, 10 mg/ml)

Storage

Store at 0-5ºC

Required Equipment

- Flow cytometer (488 nm laser, green emission filter)
- Fluorescence microscope (blue exitation filter, green emission filter)
- Incubator
- Micropipette(20 µl, 1,000 µl)

Staining procedure

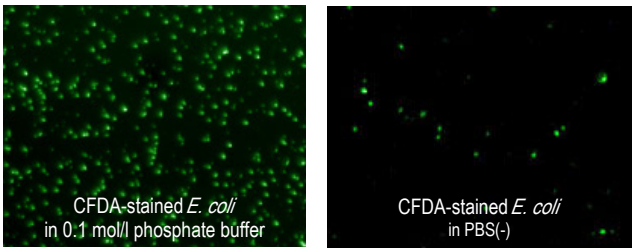
1. Allow CFDA solution to stand at room temperature for 30 minutes for thawing.
   The solution should be protected from light.
2. Resuspend the organism with an appropriate buffer (phosphate buffer, saline, etc) [a] and adjust the number of cells to 10^6 cells/ml (flow cytometry) or 10^8–10^9 cells/ml (microscopy).
3. Add CFDA solution into the 1 ml of microbial cell suspension and vortex gently to mix.
   Refer to the conditions in the following table.

	Microscopy	Flow cytometry
CFDA solution	15 µl	5 µl

4. Incubate the microbial cells at 37 ºC for 5 minutes[b].
5. Fix the microbial cells by adding of formaldehyde (1−4 % final concentration).
6. Remove the buffer by filtration or centrifugation, and resuspend the cells with the buffer.
7. Analyze the stained cells by flow cytometer or under a microscope.

a) Gram-negative bacteria tend to exhibit lower fluorescence intensity than Gram-positive bacteria, because of their cell structure (outer membrane impedes penetration of CFDA). Thus, the following buffer can be recommended.
0.1 mol/l-Phosphate buffer (pH8.5, 5%(w/v)-NaCl, 0.5 mmol/l-EDTA disodium salt)

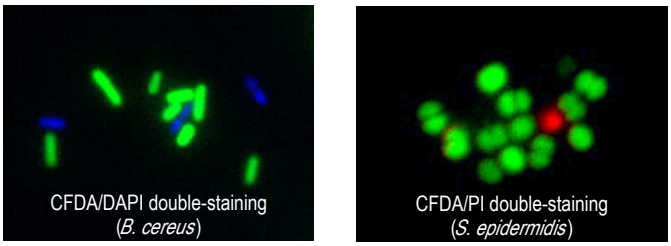
b) If sensitivity of CFDA staining is insufficient, increase the incubation time.



CFDA staining efficiency is increased by using 0.1 mol/l-phosphate buffer.

Doublestaining (Optional)

-Bacstain- DAPI solution and *-Bacstain-* PI solution can be applied as counterstaining dye.



Number of Assays

Using the protocol recommended herein, kit can perform at least 100 tests by flow cytometry or microscopy.

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.

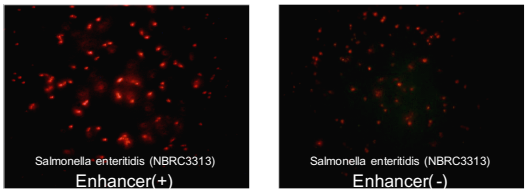
2) M. Kawai, N. Yamaguchi and M. Nasu, "Rapid enumeration of physiologically active bacteria in purified water used in the pharmaceutical manufacturing process ", *J. Appl. Microbiol.*, **1999**, *86*, 496.

3) T. Someya *et al.*, " Fluorescence Direct Count of Bacteria in Various Manures and Composts as Compared with Plate Count(Program for 2005 Annual Meeting of Japanese Society of Soil Science and Plant Nutrition)", *Journal of the science of soil and manure, Japan*, **2005**, *76*(4), 401.

Relevant products

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

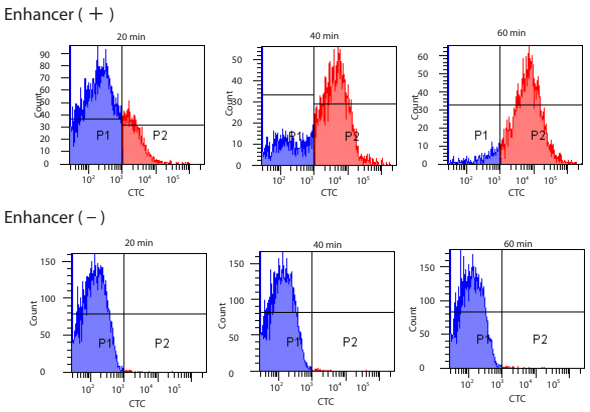
CTC has been used by many researchers to evaluate the microbial respiratory activity.
-Bacstain- CTC Rapid Staining Kit allows quick and high-sensitivity CTC-staining.



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 conditions.



Upper figures :
with Enhancing reagent

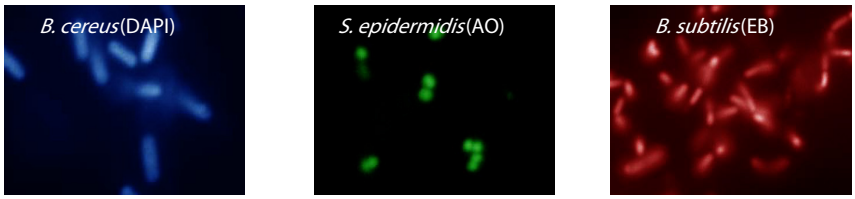
Lower figures :
without Enhancing reagent

X-axis:
Fluorescence intensity of CTC-formazan

CTC staining of *Candida albicans* in flow cytometry

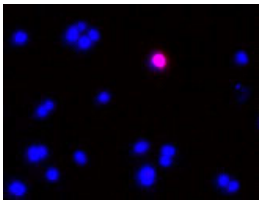
-Bacstain- DAPI solution, AO solution, EB solution

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



-Bacstain- PI solution

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



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) 500/520(dsDNA)	100
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 infomation, please contact Dojindo technical service.

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