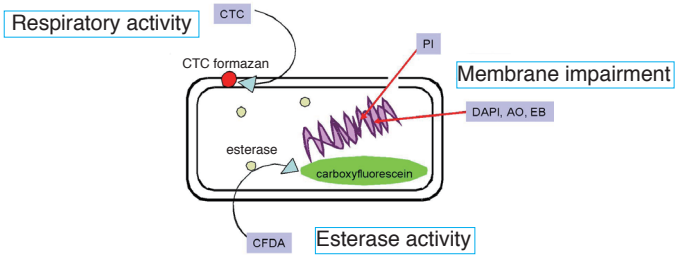
-Bacstain- CTC Rapid Staining Kit (for Microscopy) Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/bs02.pdf

Introduction

-Bacstain- series offer several kinds of bacterial fluorescence staining dye and can be applied for microbial cell viability assay in different principles.
CTC has been used by many researchers to evaluate the microbial respiratory activity.
-Bacstain- CTC Rapid Staining Kits allows quick and high-sensitivity CTC-staining.



Kit contents

CTC (5-cyano-2,3-ditolyl tetrazolium chloride) 10 mg × 3
Enhancing reagent B 500 µl (aqueous solution) × 1

Storage

Store at 0-5ºC

Required Equipment

- Incubator
- Micropipette (20 µl, 1,000 µl)
- Fluorescence microscope (blue excitation filter, red emission filter)

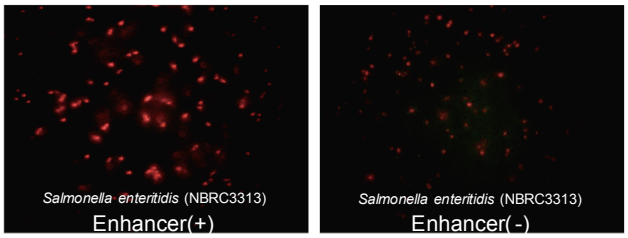
Staining procedure

1. Add 750 µl of distilled water to one CTC tube and vortex gently to dissolve (final concentration 50 mmol/l) [a].
2. Resuspend the organism with PBS(-) or saline and adjust the number of cells to 10^8-10^9 cells/ml (microscopy) [b].
3. Add each reagent into the 1 ml of microbial cell suspension and vortex gently to mix.
   Refer to the conditions in the following tables.

	CTC solution	Enhancing reagent B
Microscopy	20 µl	5 µl

4. Incubate the microbial cells at 37 ºC for 30 min[c].
5. Observe the stained microbial cells under a fluorescent microscope.

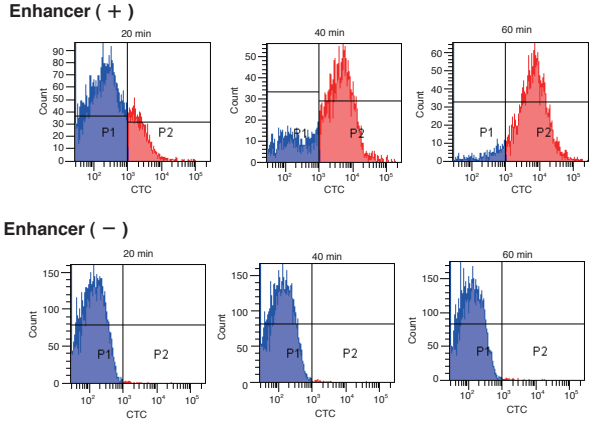
a) This solution is stable at -20ºC for 2 weeks.
b) Since remaining culture medium in the sample undergoes unspecific colored-reaction, it should be duly removed.
c) When CTC-staining is insufficient, add extra CTC solution or increase the incubation time. In this case, CTC solution should be limited less or equal to 100 µl/sample.



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.



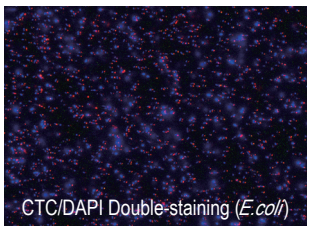
Upper figures : with Enhancing reagent
Lower figures : without Enhancing reagent

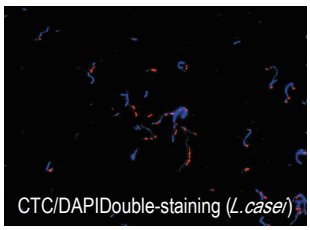
X-axis:
Fluorescence intensity of CTC-formazan

CTC staining of *Candida albicans* in flow cytometry

Counterstaining (Optional)

-Bacstain- DAPI solution is utilized for the nucleotide staining of bacteria either with membrane impairment or with intact membrane. Since CTC only stains viable aerobic cells, double staining using CTC and DAPI enable to identify viable aerobic cells among the entire cell population. Formaldehyde fixation (1–4% final concentration) is not required for the DAPI staining. However, if fixation is necessary for the downstream experiment or if the formaldehyde fixation is a standard protocol to prepare samples, it can be done in between CTC staining and DAPI staining. In this case, please add 1 µl of DAPI solution to CTC-stained cell suspension (approx. 1 ml) and then incubate the sample at room temperature for 5 min.


CTC/DAPI Double-staining (*E.coli*)


CTC/DAPIDouble-staining (*L.casei*)

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.

References

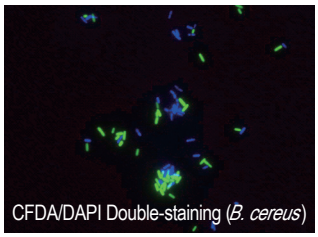
1) A.Hiraishi and N.Yoshida, "An Improved Redox Dye-Staining Method Using 5-Cyano-2, 3-Ditoryl Tetrazolium Chloride for Detection of Metabolically Active Bacteria in Activated Sludge", *Microbes Environ.*, **2004**, *19*(1), 61.

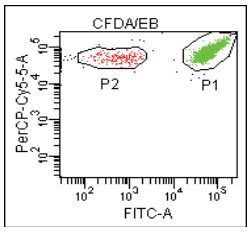
2) A. Kitaguchi, N. Yamaguchi and M. Nasu, "Enumeration of Respiring Pseudomonas spp. in Milk within 6 Hours by Fluorescence In Situ Hybridization Following Formazan Reduction", *Appl. Environ. Microbiol.*, **2005**, *71*(5), 2748.

Relevant products

-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.

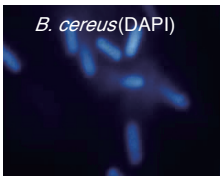

CFDA/DAPI Double-staining (*B. cereus*)

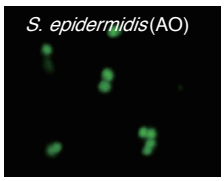


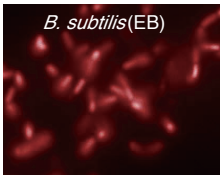
Double-staining of *S.epidermidis* (CFDA/EB)
X-axis : Fluorescence intensity of CFDA
Y-axis : Fluorescence intensity of PI

-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.

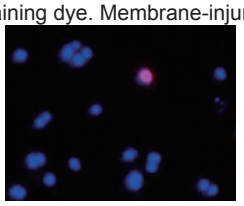

B. cereus(DAPI)


S. epidermidis(AO)


B. subtilis(EB)

-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) 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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