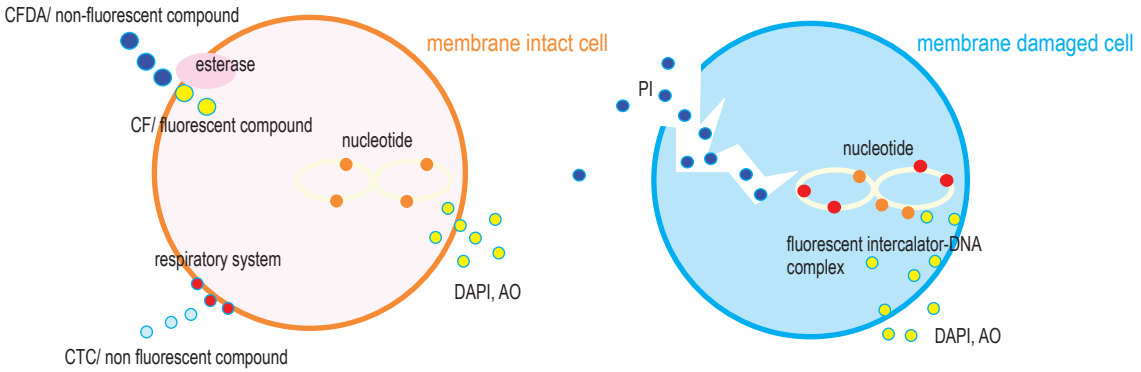
Bacteria Staining

Product Description

There are several ways to detect bacteria such as from agar plate cultivation to bacteria specific DNA amplification. Fluorescent staining using CTC is one of the methods used to detect viable bacterial cells. The advantage of this method is very quick detection and the possibility of VNC (viable but culturable) bacterial cell detection.
CTC is a tetrazolium salt that is converted to formazan dye by bacterial cell activity. The solid state of the formazan dye emits red fluorescence. Therefore, viable bacterial cells can be stained by CTC and are easily detected by fluorescent microscopy.CFDA also can be used for staining of viable microorganisms. CFDA is bacterial cell wall and cell membrane permeable, and hydrolized by esterase of the cell to stay inside of the cell. DAPI, AO, and EB are used for nucleotide staining and are cell wall permeable except for PI. Therefore, using DAPI and PI , it is possible to stain both membrane intact cells and membrane damaged cells simultaneously. Since PI can stain only membrane damaged cells, membrane intact cells are not stained by this compound. PI is also used for double staining coupled with CFDA.



Applications: Fluorescent microscopy, Flowcytometry

Required Materials

Devices, tools

- Incubator
- Safety cabinet
- Fluorescence microscope
- Flowcytometry
- Centrifuge
- Slide glass, cover glass, or chamber slide

Reagents

Living Bacterial Staining Dyes

-Bacstain- CTC Rapid Staining Kit for Flow cytometry (product code: BS01)
-Bacstain- CTC Rapid Staining Kit for Microscopy (product code: BS02)
-Bacstain- CFDA solution (product code: BS03)

Living and Dead Bacterial Staining Dyes

-Bacstain- DAPI solution (product code: BS04)
-Bacstain- AO solution (product code: BS05)

Dead Bacterial Staining Dye

-Bacstain- PI solution (product code: BS07)

Other Reagents

- Sterilized normal saline
- Formaldehyde
- PBS(-)

Preparation of Assay Solution

Some reagents are stable in the solution, however, some are not stable. Please follow the storage conditions for each reagent. Generally, the reagents offered in solution form are fairly stable.

Dyes Used For Living Bacteria Staining

Product name	Characteristic	Storage	Unit size
-Bacstain- CTC Rapid Staining Kit (for Flow cytometry) Code# BS01-10			100 assays
CTC	white-slightly orange powder	avoid light, refrigerate	
Enhancing Reagent A	slightly-yellowish solution	refrigerate	
-Bacstain- CTC Rapid Staining Kit (for Microscopy) Code# BS02-10			100 assays
CTC	white-slightly orange powder	avoid light, refrigerate	
Enhancing Reagent B	dark-purple solution	refrigerate	
-Bacstain- CFDA solution Code# BS03-10	colorless solution	avoid moisture, refrigerate	100 assays

Dyes Used for both Living and Dead Bacterial Staining

Product name	Characteristic	Storage	Unit size
-Bacstain- DAPI solution Code# BS04-10	pale yellow solution	avoid light, freeze	100 assays
-Bacstain- AO solution Code# BS05-10	yellow-orange solution	avoid light, freeze	100 assays

Dyes Used for Dead Bacteria Stain-

Product name	Characteristic	Storage	Unit size
-Bacstain- PI solution Code# BS07-10	orange-red solution	avoid light, freeze	100 assays

Since the DAPI, AO, and PI directly stain nucleus, these dyes are considered mutagens, so gloves, safety goggles, and masks are necessary when handling. If the product comes in contact with the skin, immediately wash with a copious amount of water.

When disposing remaining dye solution and solution contains staining dyes, follow the handling guidelines and the regulations at your institution and entrust disposal to an industrial waste disposal company. If the amount of the dye solution is fairly small and disposal rules and regulations of your institute are allowed, use a paper towel to adsorb and mix it in the plastic tubes used for the preparation of the staining dye solution to incinerate.

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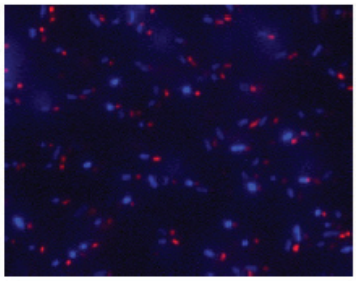
Living & Dead Bacteria Staining:

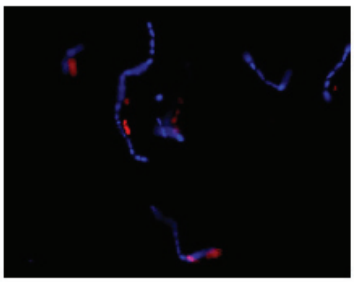
-Bacstain- CTC Rapid Staining Kit, *-Bacstain-* DAPI solution

Introduction

-Bacstain- CTC Rapid Staining Kit (used for fluorescent staining living cells) and *-Bacstain-* DAPI solution(used for fluorescent staining of living and dead cells) for simultaneous staining of living and dead cells.



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



CTC/DAPI Double-staining (*L.casei*)

Required Materials

Devices, tools

- Incubator
- Safety cabinet
- Fluorescent microsco (UV exitation light for DAPI, Blue or Green exitation light for CTC)
- Slide glass, cover glass
- Micropipette (1-10 μl, 100-1000 μl)

Reagents

- *-Bacstain-* CTC Rapid Staining Kit (for Microscopy) (product code: BS02)
  Kit contents
  CTC: 10 mg/ tube 3 vials
  Enhancing reagent B: 1 vial

- *-Bacstain-* DAPI solution (product code: BS04)
  ⚠ Store at 5°C and protect from light.

- PBS(-)

⚠ DAPI may be mutagenic, so wear gloves, safety goggles, and mask when handling. If it comes in contact with your skin, immediately wash with a copious amount of running water.

When disposing remaining dye solution and solution contains staining dyes, follow the handling guidelines and the regulations at your institution and entrust disposal to an industrial waste disposal company. If the amount of the dye solution is fairly small and disposal rules and regulations of your institute are allowed, use a paper towel to adsorb and mix it in the plastic tubes used for the preparation of the staining dye solution to incinerate.

Staining Procedure (preparing samples for use with a fluorescent microscope)

Procedure

Add 750 μl of distilled water to one CTC tube and vortex gently to dissolve (final concentration: 50 mM) [a].

Resuspend the organisms with PBS(-) or saline and adjust the number of cells to 10^6 cells/ml (flow cytometry) or 10^8-10^9 cells/ml(microscopy) [b].

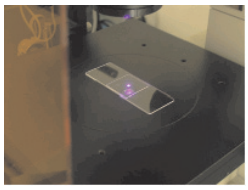
Add each reagent into the 1 ml of microbial cell suspension and vortex gently to mix[c]. *Refer to the conditions in the following table

	CTC solution	Enhancing reagent B
Microscopy	20 μL	5 μL

Incubate the microbial cells at 37°C for 30 min [d].

Add 1ml of DAPI solution to CTC-stained cell suspension and incubate at room temperature for 5-10 min.

Analyze the stained cells under a microscope.



Precautions & Tips

a) This solution is stable at -20°C for 2 weeks.

b) Since remaining culture medium in the sample undergoes unspecific colored-reaction, it should duly be 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.

d)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.

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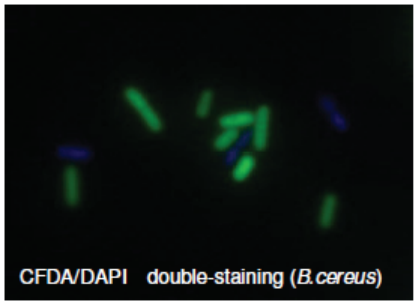
Living & Dead Bacteria Staining:

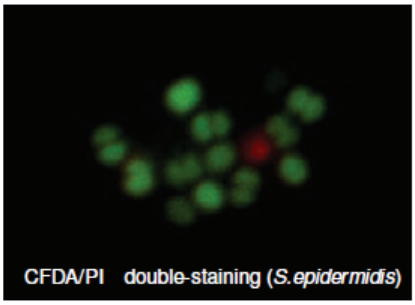
-Bacstain- **CFDA solution,** *-Bacstain-* **DAPI solution,** *-Bacstain-* **PI solution**

Introduction

-Bacstain- CFDA solution (used for fluorescent staining living bacteria) and *-Bacstain-* DAPI solution (used for fluorescent staining of living and dead bacteria) or *-Bacstain-* PI solution (used for fluorescent staining of dead bacteria) can be utilized for simultaneous staining of living and dead cells.



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



CFDA/PI double-staining (*S.epidermidis*)

Required Materials

Devices, tools

- Incubator
- Safety cabinet
- Fluorescent microsco (UV exitation for DAPI, Blue exitation for CFDA, Green exitation for PI)
- Slide glass, cover glass
- Micropipette (1-20 μl, 100-1000 μl)

Reagents

- *-Bacstain-* CFDA solution (product code: BS03)
- *-Bacstain-* DAPI solution (product code: BS04)

⚠ Store at 5°C and protect from light.
CFDA solution is easily hydrolized by moisture. Tightly close the cap after each use.

- *-Bacstain-* PI solution

⚠ Store at -20°C

- PBS(-)

⚠ DAPI and PI may be mutagenic, so wear gloves, safety goggles, and mask when handling.
If it comes in contact with your skin, immediately wash with a copious amount of running water.

When disposing remaining dye solution and solution contains staining dyes, follow the handling guidelines and the regulations at your institution and entrust disposal to an industrial waste disposal company. If the amount of the dye solution is fairly small and disposal rules and regulations of your institute are allowed, use a paper towel to adsorb and mix it in the plastic tubes used for the preparation of the staining dye solution to incinerate.

Living & Dead Bacteria Staining:

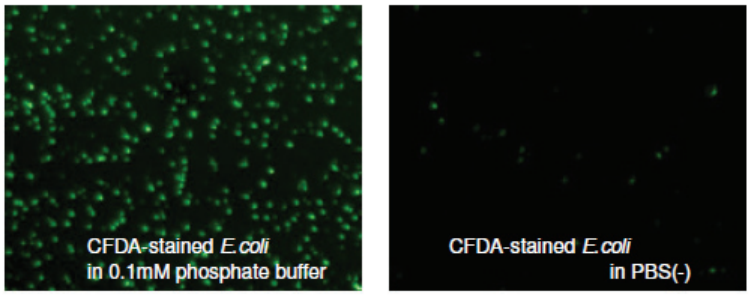
-Bacstain- **CFDA solution,** *-Bacstain-* **DAPI solution,** *-Bacstain-* **PI solution**

Staining Procedure (preparing samples for fluorescent microscope)

Procedure	Precautions & Tips
Allow CFDA solution to stand at room temperature for 30 min for thawing.	Solution should be protected from moisture.
Resuspend the organism with an appropriate buffer (phosphate buffer, saline,etc) and adjust the number of cells to 10^6cells/ml (flow cytometry) or 10^8-10^9cells/ml (microscopy).	
Add CFDA solution into the 1 mL of microbial cell suspension and vortex gently to mix. * Refer to the conditions in the following table.	
Incubate the sample at 37°C for 5 min .	If CFDA staining is insufficient, increase the incubation time.
Fix the microbial cells by addition of formaldehyde (1-4 % final concentration).	
Remove the buffer by filtration or centrifugation, and resuspend the cells with the buffer.	
Analyze the stained cells under a microscope or by flow cytometer.	

	Microscopy	Flow cytometry
CFDA solution	15 µL	5 µL

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 mM-Phosphate buffer (pH 8.5, 5%(w/v)-NaCl, 0.5 mM-EDTA disodium salt)



CFDA staining efficiency is increased by using 0.1 mM-Phasphate buffer.

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