-*Bacstain*- Bacterial Viability Detection Kit – CTC/DAPI

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/bs09.pdf

General Information

-Bacstain- Bacterial Viability Detection Kits are series of products for fluorescent double-staining of bacteria. By combining different types of fluorescent stain, staining images can be acquired for each index (membrane impairment, respiratory activity, and esterase activity). Bacterial viability is generally assessed by colony formation in nutrient-agar medium. However, this requires a long culturing time, and it is hard to recognize the growth of viable but nonculturable (VNC) bacteria.
However, fluorescent staining does not require bacterial culture, and enables viability assessment by rapid and simple protocols.
-Bacstain- Bacterial Viability Detection Kit – CTC/DAPI uses respiratory activity of bacteria as an index. CTC produces formazan dye upon reduction by NAD(P)H formed by bacterial respiratory activity, and hence selectively stains active bacteria. DAPI is a minor groove binder specific to the AT sequence of DNA and permeates into bacteria to stain nucleic acids regardless of membrane damage.
Thus, this kit can be used to measure the ratio of bacteria with respiratory activity to total bacteria on analysis of the fluorescent images from each stain.

Code	Product name	Combination
BS08	*-Bacstain-* Bacterial Viability Detction Kit - DAPI/PI	Nucleic acids/Membrane impairment
BS09	*-Bacstain-* Bacterial Viability Detction Kit - CTC/DAPI	Respiratory activity/Nucleic acids
BS10	*-Bacstain-* Bacterial Viability Detction Kit - CFDA/PI	Esterase activity/Membrane impairment

Kit Contents

CTC (5-cyano-2,3-ditolyl tetrazolium chloride)	10 mg × 4
Enhancing Reagent	700 µl × 1
DAPI Solution	25 µl × 4

Conditions

Store at 0-5 °C

Required Equipment and Materials

- Incubator
- Micropipettes (20 µl and 1,000 µl)
- PBS(-) or saline
- Sterilized water
- Fluorescence microscope

Precaution

Centrifuge the tube briefly before opening the cap because the contents may be on the tube wall or cap.

General Protocol

Each reagent included in this kit is sufficient for 100 samples.

1) Equilibrate CTC, Enhancing Reagent and DAPI Solution at room temperature by leaving the tubes for 30 min in the dark. Add 750 µl of distilled water to one CTC tube and vortex to mix well to dissolve (final concentration of CTC solution: 50 mmol/l).[a)]
   NOTE: Since DAPI may be carcinogenic, please be careful in its handling/disposing.
2) Adjust the bacterial suspension to 10^8–10^9 bacteria/ml with PBS(-) or saline.
3) Transfer 1 ml of bacterial suspension to a microtube.[b)]
4) Centrifuge the suspension (5,000 x g, 5 min).
5) Remove the supernatant, add 1 ml of PBS(-) or saline and resuspend the bacteria.
6) Repeat steps 4) and 5) twice.
7) Add CTC solution and Enhancing Reagent to the 1 ml of bacterial suspension and vortex to mix well (final concentration of CTC solution: 1 mmol/l).

CTC solution	Enhancing Reagent
20 µl	5 µl

8) Incubate the bacterial suspension at 37°C for 30min. [c)]
9) Add 1µl of DAPI Solution to the 1-ml bacterial suspension and vortex to mix well (final concentration of DAPI: 2.8 µmol/l).
10) Incubate the bacterial suspension at room temperature for 5 min.

Componet name	Maximum Ex/Em (nm)	Recommended filter	Excitation	Emission
CTC solution	430,480/630	Texas Red	520–600 nm	555–705 nm
DAPI Solution	360/460	DAPI	320–400 nm	410–510 nm

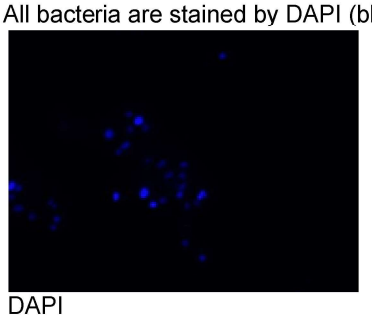
a) This solution is stable at -20°C for 2 weeks.
b) Since remaining culture medium in the sample undergoes unspecific colored-reaction, it should be duly removed.
c) When the CTC-staining is insufficient, add extra CTC solution or increase the incubation time.
In this case, CTC solution should be limited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

Experimental example-1

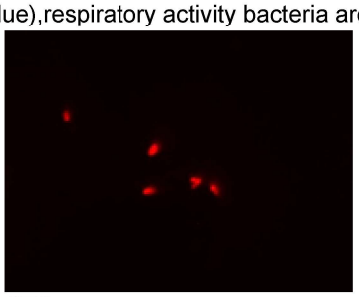
Double-staining of *Staphylococcus aureus* (a Gram-positive bacterium)

1) Two test-tubes containing *Staphylococcus aureus* in 5 ml of liquid medium (TSBYE) were prepared and cultured at 37°C for 14–16 h. One tube was used as a live bacterial sample, and the other was autoclaved at 121°C for 30 min to prepare a sterilized sample.
2) The samples prepared in step 1 were diluted with PBS(-) to adjust the bacterial suspension to 10^8–10^9 bacteria/ml, then the live and sterilized samples were mixed at a ratio of 7:3.
3) One milliliter of bacterial suspension was transferred to a microtube.
4) The bacterial suspension was centrifuged at 5,000 x *g* for 5 min.
5) The supernatant was removed and 1 ml of PBS(-) was added to the tube.
6) Steps 4) and 5) were repeated twice.
7) Twenty microliter of CTC solution and five microliter of Enhancing Reagent were added to the bacterial suspension and mixed well.
8) The bacterial suspension was incubated at 37°C for 30min.
9) One microliter of DAPI Solution was added to the bacterial suspension and mixed well.
10) The bacterial suspension was Incubated at room temperature for 5 min.
11) The bacteria were observed under an epi-illumination fluorescence microscope.

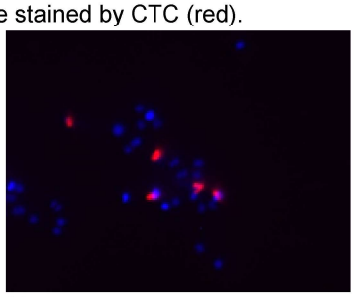
All bacteria are stained by DAPI (blue),respiratory activity bacteria are stained by CTC (red).



DAPI
Excitaition filter 320–400 nm
Emission filter 410–510 nm



CTC
Excitaition filter 520–600 nm
Emission filter 555–705 nm



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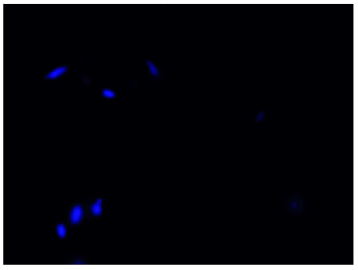
Experimental example-2

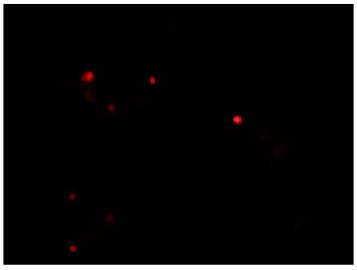
Double-staining of *Escherichia coli* (a Gram-negative bacterium)

1) Two test-tubes containing Escherichia coli in 5 ml of liquid medium (TSBYE) were prepared and cultured at 37°C for 14–16 h. One tube was used as a live bacterial sample, and the other was autoclaved at 121°C for 30 min to prepare a sterilized sample.
2) The samples prepared in step 1 were diluted with PBS(-) to adjust the bacterial suspension to 10^8–10^9 bacteria/ml, then the live and sterilized samples were mixed at a ratio of 1:1.
3) One milliliter of bacterial suspension was transferred to a microtube.
4) The bacterial suspension was centrifuged at 5,000 x *g* for 5 min.
5) The supernatant was removed and 1 ml of PBS(-) was added to the tube.
6) Steps 4) and 5) were repeated twice.
7) Twenty microliter of CTC solution and five microliter of Enhancing Reagent was added to the bacterial suspension and mixed well.
8) The bacterial suspension was Incubated 37°C for 30min.
9) One microliter of DAPI Solution was added to the bacterial suspension and mixed well.
10) The bacterial suspension was Incubated at room temperature for 5 min.
11) The bacteria were observed under an epi-illumination fluorescence microscope.

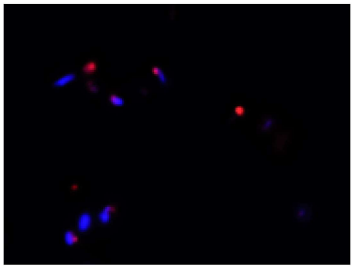
All bacteria are stained by DAPI (blue) respiratory activity are bacteria stained by CTC (red).



DAPI
Excitaition filter 320–400 nm
Emission filter 410–510 nm



CTC
Excitaition filter 520–600 nm
Emission filter 555–705 nm



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

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