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

Acridine orange (AO) has been used as total direct counting dye in the environmental bacterial research and forms a complex with double-stranded DNA to emit green fluorescence. AO also forms a complex with single-stranded DNA or RNA to emit red fluorescence.

**Mechanism of RNA and ssDNA Staining**

AO aqueous solution (75 μl × 4, 1 mg/ml)

Store at -20°C.

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

- This 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.

1. Allow AO solution to stand at room temperature for 30 min for thawing. Solution should be protected from light 

2. Resuspend the organisms with PBS(-) or saline and adjust the number of cells to $10^6$ cells/ml (flow cytometry) or $10^7$-$10^8$ cells/ml (microscopy).

3. Add 3 μl of AO solution into the 1 ml of microbial cell suspension and vortex gently to mix. Formaldehyde-fixation (1–4% final concentration) can be recommended if necessary.

4. Incubate the microbial cells at room temperature for 5 min.

5. Analyze the stained cells by flow cytometer or under a microscope.

" Since AO may be carcinogenic, please be careful in its handling/disposing.

# Kit contents
- AO aqueous solution (75 μl × 4, 1 mg/ml)

# Storage
Store at -20°C.

# Required Equipment
- Fluorescence microscope (blue excitation filter, green or red emission filter)
- Flow cytometer (488 nm laser, green or red emission filter)
- Micropipette (20 μl, 1,000 μl)

# Precaution
- This 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.

1. Allow AO solution to stand at room temperature for 30 min for thawing. Solution should be protected from light 

2. Resuspend the organisms with PBS(-) or saline and adjust the number of cells to $10^6$ cells/ml (flow cytometry) or $10^7$-$10^8$ cells/ml (microscopy).

3. Add 3 μl of AO solution into the 1 ml of microbial cell suspension and vortex gently to mix. Formaldehyde-fixation (1–4% final concentration) can be recommended if necessary.

4. Incubate the microbial cells at room temperature for 5 min.

5. Analyze the stained cells by flow cytometer or under a microscope.

Since AO may be carcinogenic, please be careful in its handling/disposing.

# Staining Procedure

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


**Relevant Products**

- **Bacstain- CTC Rapid Staining Kit (for Flow cytometry)**
  - 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.**

  ![CTC staining efficiencies compared in with or without enhancing reagent condition.](image)

- **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)](image)

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

  ![B. cereus (DAPI)](image)

- **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)](image)

<table>
<thead>
<tr>
<th>Products</th>
<th>Code</th>
<th>Maximum Ex/Em(nm)</th>
<th>Number of assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC Rapid Staining Kit (for Flow</td>
<td>BS01</td>
<td>430, 480/630</td>
<td>100</td>
</tr>
<tr>
<td>cytometry)</td>
<td></td>
<td></td>
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<tr>
<td>CTC Rapid Staining Kit (for Micro</td>
<td>BS02</td>
<td>430, 480/630</td>
<td>100</td>
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<tr>
<td>scopey)</td>
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<td></td>
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<tr>
<td>CFDA solution</td>
<td>BS03</td>
<td>493/515</td>
<td>100</td>
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<td>DAPI solution</td>
<td>BS04</td>
<td>530/620</td>
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<td>AO solution</td>
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<td>420–460/630–650(dsDNA)</td>
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<tr>
<td>PI solution</td>
<td>BS07</td>
<td>530/620</td>
<td>100</td>
</tr>
</tbody>
</table>

These products were developed by joint-research with Fukuoka Industrial Technical Center in Japan.

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If you require an assistance, please contact Dojindo customer service.

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