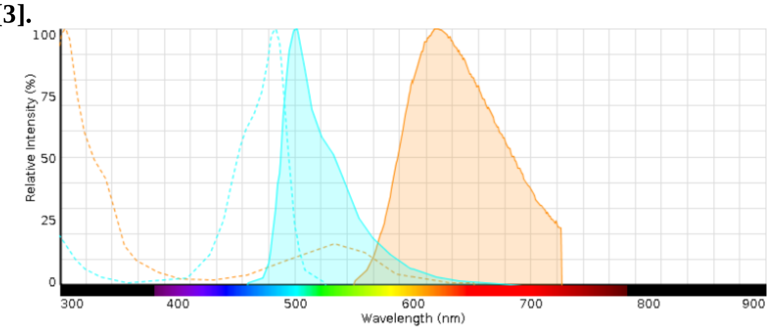
Protocol: Bacterial Viability Fluorescent Staining

For visualization of bacterial viability using SYTO® 9 and propidium iodide nucleic acid stains.

About

This method utilizes a two-color fluorescence assay to determine the viability of both gram-positive and gram-negative bacteria based on membrane integrity [1, 2]. Alone, the SYTO® 9 green fluorescent nucleic acid stain stains all bacteria, regardless of membrane integrity. The red-fluorescent propidium iodide, however, only stains bacteria with damaged membranes. When used in conjunction, propidium iodide overwhelms green fluorescence in bacteria with damaged membranes, resulting in red fluorescence. Therefore, only bacteria with intact membranes continue to fluoresce green. The excitation/emission maxima for SYTO® 9 and propidium iodide are 485/498nm and 535/617nm, respectively [1].

Figure 1. Fluorescence spectra of SYTO® 9 (cyan) and propidium iodide (orange). Dotted lines indicate excitation, whereas solid lines indicate emission [3].



Membrane integrity generally indicates cell viability, however, under certain conditions, it is possible for cells with compromised membranes to recover and reproduce, and for cells with intact membranes to be incapable of reproduction [1]. Despite this caveat, this protocol utilizes SYTO® 9 and propidium iodide nucleic acid stains because this two-color assay has been found to be one of the most effective compared to other viability visualization options. The same study found that SYTO® 9/propidium iodide staining saw fading after 15-20 minutes [4].

Caution: Propidium iodide is a potential mutagen. The mutagenicity and/or toxicity of SYTO® 9 is currently unknown. Therefore, handle both reagents with care, especially the DMSO stock solutions, as DMSO facilitates organic molecule breach of tissue. Wear double-gloves [1, 2].

Materials

- SYTO® 9 green fluorescent nucleic acid stain– 3.34mM in DMSO
- Propidium iodide– 20mM in DMSO
- Bacterial sample
- 0.2µm filter-sterilized H_2O
- Glass petri dishes or microcentrifuge tubes
- Fluorescence microscope with appropriate filters (see Table 1)

Table 1. Common filters that facilitate visualization of SYTO® 9 and propidium iodide stained cells [2].

Omega Filters [5]	Chroma Filters [6]	Utility
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous visualization of SYTO® 9 and propidium iodide stains.
XF22, XF23	31001, 41001	Bandpass filters for viewing SYTO® 9 alone.
XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004	Bandpass filters for viewing propidium iodide alone.

Protocol

1. Prep bacterial samples
   1.1. Grow bacterial cultures to appropriate degree in appropriate medium.
   1.2. Gently wash samples with filter-sterilized H_2O to remove excess medium and planktonic cells.
   1.3. Place samples in individual glass petri dishes or sterile microcentrifuge tubes.
2. Prep reagents
   2.1. Thaw SYTO® 9 and propidium iodide solutions shortly before use.
   2.2. Remove desired total quantities and immediately return stocks to ≤-20ºC freezer.
   2.3. Add a 1:1 ratio of SYTO® 9 stain and propidium iodide to filter-sterilized H_2O to yield a 0.6% solution, mix thoroughly [7].
   2.4. Place stain mixture into container capable of containing sample.

- Use prepared staining solution within the day or discard.

3. Stain sample
   3.1. Gently dunk sample into stain mixture, taking care to avoid disturbing any biofilm.
      - Stain sample before biofilm dries out.
   3.2. Incubate: 15min, RT, protected from light.
   3.3. Gently rinse sample with filter-sterilized H_2O, removing all excess stain.
   3.4. Flip sample onto a dish with a glass bottom.
      - Immediately proceed to visualization, as fluorescence will quickly fade [4].
4. Visualize sample via confocal microscopy
5. Disposal of materials
   5.1. Pour all solutions containing the SYTO® 9 stain and/or propidium iodide through activated charcoal.
   5.2. Dispose of the filtered solution.
   5.3. Incinerate charcoal to destroy dyes.
   5.4. Autoclave samples and place in traditional waste.

References

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