Propidium Iodide (PI) Staining Method

Dr. Steven Wilhelm

Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Modified from J Mol Biol 13,269 (1965)

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Protocol

Sample Preparation

Step 1.

Make a sample by adding 25% glycerol to cells

REAGENTS
Glycerol MRGE-4002 by growcells.com

NOTES
Alyssa Alsante 06 Jun 2017

Samples can be stored at -20ºC for 30 days.

Staining Solutions Preparation

Step 2.

Add DAPI at 50 µg/mL in dH_2O

Staining Solutions Preparation

Step 3.

Add PI at 500 µg/mL in dH_2O

Staining Solutions Preparation

Step 4.

Filter sterilize with 0.2 µm Millipore filter and store in the dark at 4ºC

Slide Preparation

Step 5.

Dilute cells appropriately according to your sample

Step 6.

Add DAPI at a final concentration of 5 µg/mL

Step 7.

Add PI at a final concentration of 3 µg/mL

Step 8.

Incubate in the dark for 30 min

DURATION
00:30:00

Step 9.

Filter sample onto a 0.2 µm black polycarbonate filter under low vacuum (<15 mmHg)

Step 10.

Mount the filter onto a glass slide with low fluorescence immersion oil

Step 11.

View the sample under the Leica DM4000-6000 epifluorescent microscope under the Texas Red N3 filter at green excitiation = 546 nm and emission = 600 nm