

Ethidium Homodimer I

Table 1 - Materials Needed		Most Items Available from ION Biosciences
Name	Volume	Description/Purpose
Ethidium Homodimer I - Available in Two Forms:		
⇒ Dry - 1 mg Vials	1 x 1 mg Vial	Membrane Impermeable Dye Indicator
⇒ Solution - 2 mM in DMSO/H ₂ O	20 µL	
DMSO ¹	250 µL	Solvent for Dissolution of Dye
Water	750 µL	Solvent for Dissolution of Dye
Assay Buffer - We Suggest One of the Following:		
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	10 mL	Buffer for Preparing Dye Loading Solutions
⇒ 1X Phosphate Buffered Saline	10 mL	

Description

Ethidium homodimer I (EthD-I) is a membrane-impermeable, high-affinity, nucleic acid stain that is excluded by viable cells with intact cell membranes. When membrane integrity is compromised, a hallmark of dead or dying cells, EthD-I enters the cell and binds DNA, which results in a >30-fold enhancement in bright red, nuclear fluorescence (Ex/Em 528 nm/617 nm).

Laboratory Procedures - General Considerations

1. Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment if conducting additional assays with Ethidium Homodimer I.
2. In addition to reagents, a microscope and fluorescence plate reader that is capable of providing an excitation source at ~ 530 nm and measuring emission at ~620 nm is required.
3. Optimal dye concentrations will vary depending on cell type and application. Recommended dye concentrations range between 0.1 µM and 10 µM.
4. Aqueous DMSO solutions of EthD-I are generally stable to multiple freeze-thaw cycles, but unused solution must be stored at -20°C.
5. Dead cell controls can be prepared by applying 0.1% saponin or 0.1 - 0.5% digitonin to live cells for 10 min.
6. Cytotoxic events that do not affect membrane permeability will not be accurately measured using EthD-I.

Plate Reader

1. Seed cells in a 96-well (or 384-well) plate and treat with test compounds of your choosing prior to staining.
2. Remove Ethidium Homodimer I (EthD-I) from freezer and allow the dye to warm to room temperature.
3. Optional: prepare dead cells by incubating with 0.1% saponin or 0.1-0.5% digitonin solutions for 10 minutes.
4. Proceed directly to **step 6** if using EthD-I, 2 mM in DMSO/Water solution. Otherwise, make a 1:3 solution of DMSO:H₂O, for example by adding mixing 250 μ L of DMSO with 750 μ L of water.
5. Add 580 μ L of the DMSO/H₂O mixture to the dry EthD-I dye tube and vortex to mix completely.
6. Prepare a **dye loading solution** that contains 4 μ M EthD-I (1:500 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 20 μ L of EthD-I to 10 mL of 1X HHBSS. Vortex briefly to mix.
7. Add **dye-loading solution** prepared in **step 6** directly to cells. We recommend 100 μ L/well for a 96-well plate.
8. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
9. Measure fluorescence using a microplate reader. Use Ex/Em ~530 nm/620 nm or Texas Red© settings. See **Table 1** for recommended instrument settings.

Microscopy Assay

1. Remove Ethidium Homodimer I (EthD-I) from freezer and allow the dye to warm to room temperature.
2. Proceed directly to **step 4** if using EthD-I, 2 mM in DMSO/H₂O solution. Otherwise, make a 1:3 solution of DMSO:H₂O, for example by adding mixing 250 μ L of DMSO with 750 μ L of water.
3. Add 580 μ L of the DMSO/H₂O mixture the dry EthD-I dye tube and vortex to mix completely.
4. Prepare a **dye loading solution** that contains 4 μ M EthD-I (1:500 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 20 μ L of EthD-I to 10 mL of 1X HHBSS. Vortex briefly to mix.
5. Add sufficient volume of the **dye loading solution** prepared in **step 4** to completely cover cells.
6. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
7. Optional: Replace **dye loading solution** with fresh buffer or medium prior to imaging.
8. Image stained cells using fluorescence microscopy using Texas Red, propidium iodide, or rhodamine filters.

Flow Cytometry Assay

1. Remove Ethidium Homodimer I (EthD-I) from freezer and allow the dye to warm to room temperature.
2. Proceed directly to **step 4** if using EthD-I, 2 mM in DMSO/H₂O solution. Otherwise, make a 1:3 solution of DMSO:H₂O, for example by adding mixing 250 µL of DMSO with 750 µL of water.
3. Add 580 µL of the DMSO/H₂O mixture the dry EthD-I dye tube and vortex to mix completely.
4. Prepare a **dye loading solution** that contains 4 µM EthD-I (1:500 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 20 µL of EthD-I to 10 mL of 1X HHBSS. Vortex briefly to mix.
5. Pellet cells via centrifugation, remove supernatant, then resuspend in 100 µL of serum free medium or buffer.
6. Add 100 µL of **dye loading solution** prepared in **step 4** directly to cells.
7. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
8. Pellet cells again via centrifugation and resuspend in preferred flow cytometry buffer.
9. Analyze cells using a flow cytometer. To detect EthD-I (+) cells, use phycoethrin (PE) or PE-Texas Red settings.

Setting	Recommendation
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths ²	530 nm/620 nm
Cutoff wavelength	600 nm
Filter selection	Texas Red, Propidium Iodide, Rhodamine, phycoethrin (PE)
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

- ¹ DMSO is used to increase the stability of the EthD-I solution when frozen. Ethidium Homodimer I is fully soluble in water if DMSO is preferred not to be used. Unused EthD-I solution should be stored at -20°C.
- ² To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

Ethidium Homodimer I, 2 mM in DMSO/H₂O must be shipped frozen.

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department.

Table 2	Available Dye Indicators	Available Sizes	
		Size	Catalog #
	Ethidium Homodimer I	1 mg	5010
	Ethidium Homodimer I, 2 mM in DMSO/H ₂ O	0.5 mL	5020
	1X HEPES-buffered Hanks Balanced Salt Solution (HHBSS)	100 mL	7001