LIVE/DEAD® Sperm Viability Kit (L-7011)

Quick Facts

Storage upon receipt:

- –20°C
- Protect from light

Note: Component B (propidium iodide) may precipitate upon freezing. After thawing, gently vortex or sonicate to dissolve the dye.

Introduction

The LIVE/DEAD® Sperm Viability Kit provides a novel fluorescence-based assay for analyzing the viability and fertilizing potential of sperm. Commonly used sperm viability assays employ mixtures of two or three dyes, including fluorescein diacetate derivatives, rhodamine 123 and reduced nucleic acid stains, which target a variety of cellular components.[1-4] Acridine orange, which fluoresces at different wavelengths when bound to DNA and RNA,[5,6] and the UV-excitable nucleic acid stain Hoechst 33342 [7,8] are also frequently used for determining sperm viability and DNA content.

The LIVE/DEAD Sperm Viability Kit, developed in cooperation with Duane Garner at the University of Nevada, Reno, contains a membrane-permeant nucleic acid stain developed at Molecular Probes (SYBR® 14 dye), and the conventional dead-cell stain, propidium iodide. The dyes provided in the LIVE/DEAD Sperm Viability Kit label cells more rapidly than conventional stains, and both dyes label DNA, thereby circumventing the ambiguity that may arise from targeting separate cellular components. In addition, using this combination of dyes, researchers can distinguish live and dead cells with visible-light excitation, avoiding the harmful effects of UV exposure.[9] When bovine semen is incubated briefly with these two stains, live sperm cells with intact cell membranes fluoresce bright green, while cells with damaged cell membranes fluoresce red.

Garner and colleagues assessed sperm viability using flow cytometry and fluorescence microscopy, though this kit can likely be adapted for use with other instrumentation. The LIVE/DEAD Sperm Viability Kit has yielded reliable results with both bovine and goat sperm and is currently being tested on human sperm. The membrane-permeant nucleic acid stain included in this kit should also provide researchers with a valuable tool for labeling and tracking live sperm, facilitating analysis of their motility and abundance in semen samples. The LIVE/DEAD Sperm Viability Kit is intended as a research tool; our Technical Assistance Department welcomes any feedback on the performance of this kit with sperm from other species. We will strive to keep the research community informed as we receive additional information.

Materials

Kit Contents

- **SYBR 14 dye** (Component A), 100 µL of a 1 mM solution in DMSO
- **Propidium iodide** (Component B), 5 mL of a 2.4 mM solution in water

When used at the recommended reagent dilutions and volumes, this kit contains sufficient material to perform ~200–1000 assays.

Storage and Handling

Stock solutions should be stored frozen at -20°C and protected from light. Allow reagents to warm to room temperature and centrifuge briefly before opening the vials. Before refreezing, seal all vials tightly. When stored properly, these stock solutions are stable for at least one year.

Note: Propidium iodide (Component B) may precipitate upon freezing. After thawing to room temperature, gently vortex or sonicate to dissolve the dye. Use propidium iodide only when it is completely free of dye crystals.

Caution: No data are available addressing the mutagenicity or toxicity of the SYBR 14 reagent (Component A). Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. Propidium iodide is known to have mutagenic effects on cells and should be handled with care. Avoid prolonged or repeated exposure and do not get in eyes, on skin or on clothing. Wash thoroughly after handling. As with all nucleic acid stains, solutions containing these reagents should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Staining Protocol

General Considerations

The following protocol is provided as an example to guide researchers in the development of their own staining procedures. This method is based on procedures developed by Garner and has been found to be reliable for discriminating live and dead bovine sperm cells. Concentrations of reagents required for optimal staining may vary depending on the density of sperm cells and other material in the sample. The phototoxicity of SYBR 14 dye (Component A) may be enhanced at concentrations higher than the 100 nM levels recommended in the protocol given below.

Note that phosphate-containing buffers may interfere with SYBR 14 dye staining. Also, preparation of dilute stock solutions of SYBR 14 dye in aqueous media may result in unaccept-

able dye loss and therefore is not recommended. If diluted solutions of SYBR 14 dye are to be stored and reused, they should be prepared in DMSO.

Both SYBR 14 dye and propidium iodide can be excited with visible-wavelength light. When bound to DNA, the fluorescence emission maxima of these dyes are 516 nm and 617 nm, respectively (Figure 1). Any of the filter sets listed in Table 1 may be used for simultaneous observation of SYBR 14 dye and propidium iodide by epifluorescence microscopy.

Staining Sperm Cells

1. Dilute semen sample in HEPES-buffered saline solution containing bovine serum albumin (10 mM HEPES, 150 mM NaCl, 10% BSA, pH 7.4).[10] Dilutions of 1:10 (goat) to 1:40 (bovine) result in acceptable cell densities. Debris can be removed by glass wool, gel filtration or other methods.

2. Prepare a 50-fold dilution of the SYBR 14 stock solution (Component A) in buffer. *Prepare aqueous dilutions immediately before use. Aqueous solutions of SYBR 14 dye should not be stored or reused.*

3. Add 5 µL of diluted SYBR 14 dye (from step 2) to a 1 mL sample of diluted semen, resulting in a final SYBR 14 concentration of 100 nM. Alternately, the SYBR 14 dye concentrate (Component A) may be diluted 10-fold in DMSO and 5 µL of this new stock solution may be added to 5 mL of diluted semen.

4. Incubate for 5–10 minutes at 36°C.

Table 1. *Characteristics of common filters suitable for use with the LIVE/DEAD Sperm Viability Kit.*

Omega Filters *	Chroma Filters *	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of SYBR 14 and propidium iodide.
XF22, XF23	31001, 41001	Bandpass filters for viewing SYBR 14 alone
XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004	Bandpass filters for viewing propidium iodide alone

* Catalog numbers for recommended bandpass filter sets for fluorescence microscopy. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).

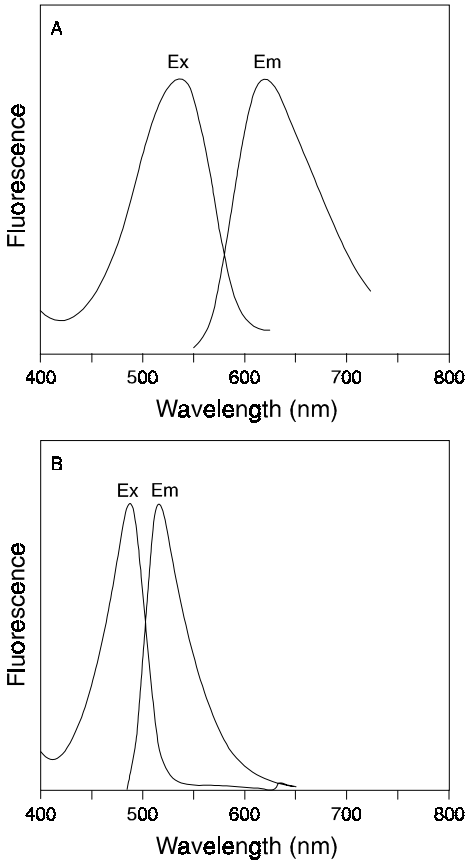

Figure 1. *Excitation (Ex) and emission (Em) spectra of the A) propidium iodide/DNA complex and B) SYBR 14 dye/DNA complex*

5. If differential staining of live and dead cells is desired, add 5 µL of propidium iodide (Component B) to the 1 mL sample of diluted semen. The final propidium iodide concentration will be 12 µM.

6. Incubate an additional 5–10 minutes.

7. Observe the sample in a fluorescence microscope equipped with any of the filter sets described in Table 1 or equivalent filters. Alternatively, the sample may be analyzed by flow cytometry.

References

1. Theriogenology 39, 1009 (1993); **2.** Gamete Res 22, 355 (1989); **3.** Biol Reprod 34, 127 (1986); **4.** J Histochem Cytochem 30, 279 (1982); **5.** Methods Cell Biol 33, 401 (1990); **6.** J Histochem Cytochem 25, 46 (1977); **7.** Cytometry 8, 642 (1987); **8.** Cytometry 1, 132 (1980); **9.** J Androl Suppl P-44, abstract #95 (Jan/Feb 1994); **10.** Gamete Res 7, 49 (1983).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
L-7011	LIVE/DEAD® Sperm Viability Kit *200-1000 assays*	1 kit

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