**SYBR-14/PI Sperm Viability Staining**

**Materials**

LIVE/DEAD Sperm Viability Kit (L-7011)

**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

**Procedure**

1. Dilute semen in 1 ml Kiev buffer solution (D+ glucose - 0.3 g, potassium chloride - 0.41 g, sodium bicarbonate - 0.21 g, sodium citrate-2 hydrate - 2.43 g, in 100 ml of deionized, sterilized water). This is an acceptable cell density.
2. Prepare a 50-fold dilution of the SYBR-14 stock solution (component A) in buffer. Prepare aqueous dilutions immediately before use.
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 tenfold in DMSO, and 5 μL of this new stock solution may be added to 5 mL of diluted semen.
4. Incubate for 5–10 min at 36 °C.
5. Add 5 μL of propidium iodide (component B) to the 1 mL sample of diluted semen.
6. Incubate an additional 5–10 min.
7. Observe the sample in a fluorescence micro- scope equipped with a fluorescein isothiocyanate (FITC) filter set or equivalent filters. Both SYBR 14 and PI were excited by 488 nm. SYBR 14 fluorescent emission was collected in the range of 515–545 nm (530/30 bandpass), whereas PI fluorescent emission was collected in the range of 670–735 nm (670 longpass [LP]).