Helixyte™ 14 [Equivalent to SYBR 14] *5 mM*

Catalog number: 17563
Unit size: 0.5 mL

Component	Storage	Amount
Helixyte™ 14 [Equivalent to SYBR 14] *5 mM*	Freeze (< -15 °C), Minimize light exposure	0.5 mL (5 mM)

OVERVIEW

Helixyte™ 14 has the same chemical structure of SYBR 14 (SYBR is the trademark of ThermoFisher). It is commonly used to monitor the viability of sperms. The proportion of living sperm in semen can be assessed by means of a dual staining technique using the stains SYBR-14 and propidium iodide (PI). Helixyte™ 14 is a nucleic acid stain, maximally absorbs at 488 nm and emits at 518 nm when bound to DNA. It stains the nuclei of living sperm bright green. Conversely, PI stains only nonmotile sperm that had lost their membrane integrity. The proportions of living and dead sperm can be determined by first staining with SYBR-14 and PI and then assessing stain uptake by flow cytometry. The proportions of living and dead sperm in mammalian semen can be readily identified through use of dual staining with SYBR-14 and PI and quantified through use of flow cytometry.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Helixyte™ 14 working solution
Dilute the 5 mM Helixyte™ 14 stock solution to 10 to 20 µM Helixyte™ 14 working solution in the buffer of your choice.

Note Prepare the working solution immediately before use.

Note The Helixyte™ 14 working solution should not be stored or reused.

Note The concentration of the Helixyte™ 14 working solution should be optimized for different cell types and conditions.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol is provided as a basic guide for the development of your own staining protocol. The concentrations of the reagents required for the optimal staining may vary depending on the density of cells (i.e. Sperm cells) and other materials in the sample.

1. Dilute samples (e.g. Semen samples) in HEPES-buffered saline solution containing bovine serum albumin (10 mM HEPES, 150 mM NaCl, 10% BSA, pH 7.4).
2. Add 5 µL of the diluted Helixyte™ 14 working solution to 1 mL of diluted samples.
3. Incubate the samples at 37 °C for 5-10 minutes.
4. Observe the samples using a fluorescence microscope with the FITC filter set. Alternatively, the samples can be analyzed by flow cytometry using the 530/30 nm filter.

DISCLAIMER

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