Cell Navigator™CDy6 Mitosis Imaging Kit

Catalog number: 22640
Unit size: 100 Tests

Component	Storage	Amount
Component A: CDy6	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Nocodazole	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Mitosis is the most defining stage of cell growth. Cell Navigator™ CDy6 Mitosis Imaging Kit is a useful tool for monitoring mitosis by visualizing lysosome dynamics. Long-term real-time visualization of mitosis had been a challenge due to the lack of photostable and low toxicity fluorescent probes. CDy6 Mitosis Imaging Kit uses cell permeable lysosome dye CDy6 which displays high sensitivity in acidic environment and exhibit bright signal in lysosomes. During mitosis, lysosomes rapidly accumulate towards nucleus, displaying a sharp increase in signal intensity that can be visualized in real-time. CDy6 does not interfere with cell cycle and can stand repeated exposure for long-term imaging.

AT A GLANCE

Protocol summary

1. Prepare cells in a 96-well plate (100 µL/well)
2. Add 10X CDy6 working solution (10 µL/well)
3. Add test compounds
4. Incubate for the desired amount of time at 37 °C
5. Image with a fluorescence microscope using a Cy3/TRITC filter set

Important
Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3/TRITC filter set
Emission	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. CDy6 stock solution (1000X)
Add 15 µL of DMSO into the vial of CDy6 (Component A) to make 1000X CDy6 stock solution.

Note Protect from light.

2. Nocodazole stock solution (1000X)
Add 50 µL of DMSO into the vial of Nocodazole (Component B) to make 1000X Nocodazole stock solution.

PREPARATION OF WORKING SOLUTION

1. CDy6 working solution (10X)
Add 1 µL of 1000X CDy6 solution in 100 µL of culture media or buffer of your choice and mix well.

Note 100 µL of 10X CDy6 working solution is enough for 10 tests in a 96-well plate format. Prepare enough 10X CDy6 working solution right before the experiment, and use promptly.

2. Optional: Nocodazole working solution (10X)
Prepare 10X Nocodazole working solution for positive control by mixing 1 µL of Nocodazole with 100 µL of culture media and mix well.

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate 1000 to 40,000 cells/well (90 µL for 96-well plate, or 22.5 µL for 384-well plate) in a tissue culture microplate with clear bottom.

2. Add 10 µL/well (96-well plate) or 2.5 µL/well (384-well plate) of 10X CDy6 working solution to each well.

3. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48 or 96 hours) in a 5% CO_2 incubator at 37 °C.

4. (Optional) For positive control, add 11 µL/well (96-well plate) or 3 µL/well (384-well plate) of 10X Nocodazole working solution to each well and incubate for 16 hours in a 5% CO_2 incubator at 37 °C. Mitotic cell population will be significantly enriched after the treatment.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density.

5. Image with a fluorescence microscope using a Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

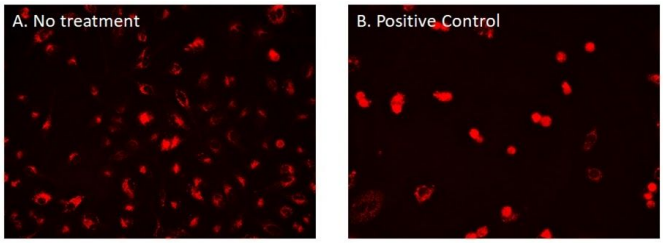

Figure 1. Images of HeLa cells stained with Cell Navigator™ CDy6 Mitosis Imaging Kit. A. Control cells with no treatment. B. Cells treated with Positive Control (Nocodazole) to enrich mitotic cell population.

DISCLAIMER

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