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Convenient Fluorimetric Assays for Detecting Apoptosis and Cytotoxicity

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Application Note for DHL™ Cell Viability/Proliferation Assay Kit (Cat#71300) and DHL™ Cytotoxicity Assay Kit (Cat#71302)

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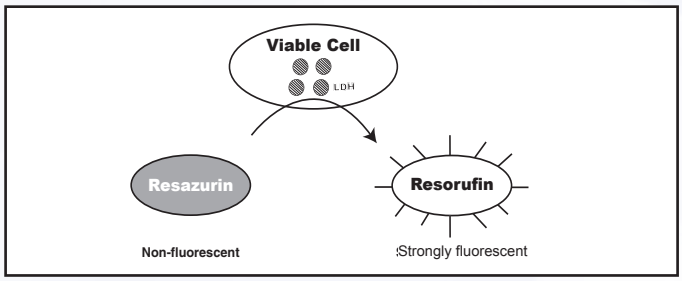
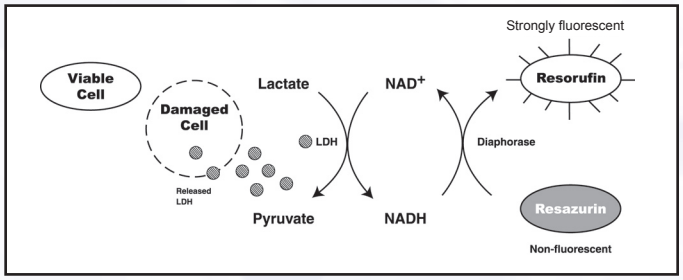
Introduction

Lactate dehydrogenase (LDH) generally exists in prokaryotic, fungal and eukaryotic cells. The measurement of cytoplasmic LDH activity is a well-accepted assay to quantify viable cell numbers and monitor cell proliferation [1]. On the other hand, the leakage of cytoplasmic LDH caused by the damage of cell membrane integrity is also a good indicator of cell death and is used to estimate cytotoxicity [2].

The DHL™ Cell Viability and Proliferation Assay Kit (Cat# 71300) and Cytotoxicity Assay Kit (Cat# 71302) utilize a sensitive fluorimetric indicator, resazurin [3], to measure LDH activity either in the cytoplasm for live cells (Scheme 1) or in the medium for dead cells (Scheme 2). Both assays are in mix and read format. The DHL™ Cell Viability and Proliferation Assay needs a single reagent-adding step and can continuously monitor cell proliferation over 24 hours. The DHL™ Cytotoxicity Assay Kit requires a single addition of reaction mixture and a 10-minute read-out. Both assays have proven to correlate very well with traditional isotope-based proliferation or cytotoxicity assays, such as [^{3}H]thymidine incorporation assay [4], and ^{51}Cr release assay [5].



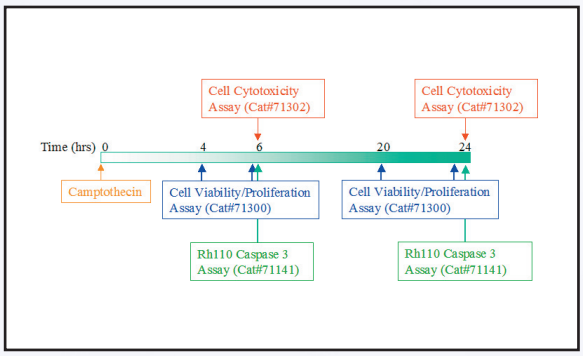
Scheme 1. DHL™ Cell Viability and Proliferation Assay Kit (Cat#71300). Dehydrogenases (e.g. LDH) in live cells continuously convert non-fluorescent resazurin to the strongly fluorescent resorufin, monitored at the emission wavelength of 590 nm with the excitation wavelength at 530-560 nm.



Scheme 2. DHL™ Cytotoxicity Assay Kit (Cat#71302). Upon cell death, the cytoplasmic dehydrogenases (e.g. LDH) are released to the surrounding culture medium. By using an enzyme-coupled reaction, the dehydrogenases in the medium will convert non-fluorescent resazurin to the strongly fluorescent resorufin, monitored at the emission of 590 nm with the excitation at 530-560 nm. Under the same assay condition, the viable cells produce negligible fluorescent signal. Therefore, the assay can be performed in a mixture population of damaged and viable cells.

Experiment Methods and Results

In current study, we use these two assay kits to measure the proliferation and toxicity effect of camptothecin on Jurkat cells. Camptothecin is a known apoptosis inducer [6]. The assay procedure is outlined in scheme 3. The data are shown in Figures 1-3.



Scheme 3: The assay procedure.
3X10[4]/well Jurkat cells were seeded in a clear-bottomed and black-walled 96-well plate. On the second day, camptothecin was added to the cells and then the cells were incubated at 37°C for 4 hrs. The cell viability and proliferation assay reagents were added to the cells and the fluorescence signal was monitored 2 hrs later. Six hours after adding camptothecin, the cytotoxicity and caspase-3 activity were analyzed using the DHL and EnzoLyte kits. Another parallel plate of Jurkat cells was incubated with camptothecin for 20 hrs and the cell viability and proliferation assay reagents were added with the signal monitored at 4 hrs later. 24 hours after adding camptothecin, the cytotoxicity and caspase-3 activity were analyzed using the DHL and EnzoLyte Kits.

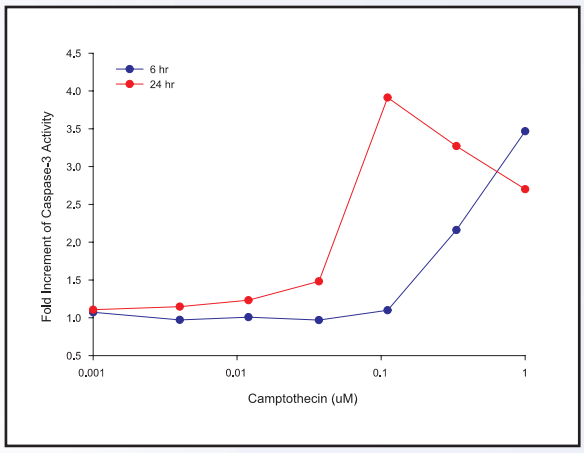


Figure 1. Apoptosis induced by camptothecin.
6 hrs and 24 hrs after adding camptothecin, caspase-3 activity was measured using the EnzoLyte™ Rh110 Caspase-3 Assay Kit (Cat# 71141). Caspase-3 activity increased after 6 hr and continuously strengthened when the incubation was prolonged to 24 hrs. But at higher camptothecin concentration, caspase-3 activity decreased at 24 hr because of increasing number of dead cells.

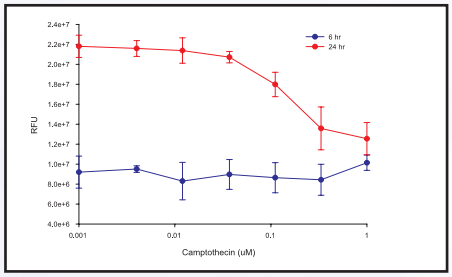


Figure 2. Growth inhibition effect of camptothecin.
The number of viable cells was determined by DHL™ Cell Vialibity/ Proliferation Assay Kit (Cat# 71300) after Jurkat cells were exposed to camptothecin for 6 and 24 hr. The growth of Jurkat was not affected significantly at 6 hr, but decreased at 24 hr.

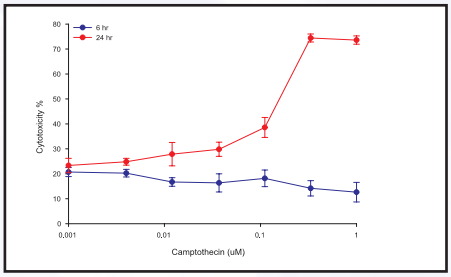


Figure 3. Cytotoxicity effect of camptothecin.
The number of dead cells was determined by DHL™ Cell Cytotoxicity Assay Kit (Cat# 71302) at 6 hr and 24 hr after camptothecin treatment. Jurkat showed little cell death at 6 hr, but increased cytotoxicity at 24 hr.

Summary

Upon camptothecin treatment, the cells undergo apoptosis within 2-8 hr. During that period, caspase-3 activity significantly increases but cell death is not significant. After extended incubation with camptothecin, the cell necrosis happens at 20-24 hr. Plasma membrane integrity is damaged and the cytoplasmic enzymes are released to the surrounding medium. The number of live cell decreases, while dead cells increases. All of above chronological biological changes can be monitored with the DHL™ and EnzoLyte™ assay kits from AnaSpec.

References

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