In Situ Apoptosis Detection Kit (Cat.# MK500)

Frequently Asked Questions: *In Situ* Apoptosis Detection Kit

DNA is fragmented during the biological process of apoptosis. This fragmentation occurs after intracellular endonucleases are activated in a regulated fashion. The *In Situ* Apoptosis Detection Set from Takara Bio allows detection of fragmented DNA histochemically by TUNEL (TdT-mediated dUTP Nick End Labeling). In this technique, fluorescein-labeled nucleotides are incorporated *in situ* onto the 3′ ends of DNA fragments, allowing histologic localization and detection of individual apoptotic cells.

Answers to frequently asked questions about the LDH Cytotoxicity Detection Kit are presented here. For additional information, refer to the product User Manual and web page.

Q1: The intensity of staining of apoptotic cells is low. How can staining intensity be improved?

A1: i) The reaction mixture may not have permeated the tissues or cells due to steric hindrance. To improve permeation, optimize the duration of Proteinase K and/or Permeabilization buffer treatment.

ii) Extend the enzymatic reaction time.

iii) Extend the antibody reaction time or the substrate coloring time.

Q2: Non-apoptotic cells are stained. How can staining specificity be improved?

A2: Non-specific binding might have occurred. Repeat the washing steps or add blocking reagent to the wash buffer (e.g., 1% (w/v) BSA, or nonfat milk).

Q3: For light microscopy, is methyl green staining always necessary? Can hematoxylin be used instead of methyl green for counterstaining?

A3: Methyl green stains nuclei. Methyl green staining can help elucidate apoptotic cells by defining the size and location of the nuclei; non-apoptotic cell nuclei will stain green, while apoptotic cells will be stained brown by DAB. It is also possible to counterstain with hematoxylin. However because hematoxylin stains nuclei violet, comparing hematoxylin staining with DAB staining may be difficult.