

For life science research only.  
Not for use in diagnostic procedures.



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# DNA Fragmentation Imaging Kit

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 **Version 05**

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**Cat. No. 06 432 344 001**

1 kit (1 × 96 tests)

**Store the kit at –15 to –25°C  
Store the kit dry and in the dark.**

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## 1. What this Product Does

**Number of Tests** The kit is designed for 96 reactions in a 96 well format microplate.

Vial/ Cap	Label	Contents/ Function
1 Blue cap	Nuclei Dye	<ul style="list-style-type: none"> <li>• Hoechst 33342</li> <li>• 1 × 40 μl</li> <li>• Fluorometry               <ul style="list-style-type: none"> <li>• Excitation maximum (<math>EX_{max}</math>) = 361 nm</li> <li>• Emission maximum (<math>EM_{max}</math>) = 486 nm</li> </ul> </li> </ul>
2 Yellow cap	Enzyme Solution	<ul style="list-style-type: none"> <li>• Terminal deoxynucleotidyl transferase from calf thymus (<i>EC 2.7.7.31</i>), recombinant in <i>E. coli</i>, in storage buffer</li> <li>• 1 × 500 μl</li> </ul>
3 Clear cap	Label solution	<ul style="list-style-type: none"> <li>• Nucleotide mixture in reaction buffer</li> <li>• 1 × 5.5 ml</li> <li>• Fluorometry               <ul style="list-style-type: none"> <li>• Excitation maximum (<math>EX_{max}</math>) = 494 nm</li> <li>• Emission maximum (<math>EM_{max}</math>) = 518 nm</li> </ul> </li> </ul>

### Storage and Stability

The product is stable until the expiration date printed on the label when stored at –15 to –25°C.

- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1	Nuclei Dye	<ul style="list-style-type: none"> <li>• Store at –15 to –25°C</li> <li>• <b>Avoid repeated freezing and thawing</b></li> <li>• Protect from exposure to light.</li> <li>• Store dry in dark.</li> </ul>
2	Enzyme Solution	<ul style="list-style-type: none"> <li>• Store at –15 to –25°C</li> <li>• <b>Avoid repeated freezing and thawing</b></li> <li>• Protect from exposure to light.</li> <li>• Store dry in dark.</li> </ul>
3	Label solution	<ul style="list-style-type: none"> <li>• Store at –15 to –25°C</li> <li>• <b>Avoid repeated freezing and thawing</b></li> <li>• Protect from exposure to light.</li> <li>• Store dry in dark.</li> </ul>

- Ⓢ The reaction mixture of enzyme and label solutions should be prepared just before use, and any remaining unused reaction mixture should be discarded.

**Additional Equipment and Reagents Required**

Additional equipment and reagents required to perform cellular analysis:

**Standard laboratory equipment:**

- Standard cell culture equipment (e.g., biohazard hoods, incubators, sterile plastic tubes)
- Standard pipettes and micropipettes
- Regular flat, clear bottom 96-well microplate

**Standard laboratory reagents:**

- Formaldehyde
- Triton-X 100
- 1× phosphate buffered saline (PBS), cell culture grade

**Cells:**

- Select subconfluent cultures in log phase for preparation of the cell cultures

**Fluorescence microscope equipped with the appropriate excitation and emission filters**

**Application**

The DNA Fragmentation Imaging Kit provides a simple and rapid method to detect apoptosis induction in mammalian cells. DNA fragmentation is measured using Terminal Deoxynucleotidyl Transferase and Fluorescein-labeled dUTP (TUNEL assay), and is evaluated based on fluorescence detection. This kit also provides nuclei staining dye for staining nuclei of both live and dead cells. This allows for the quantification of the total cell number, as well as the number and percentage of apoptotic cells.

**Assay Time for 1 × 96 well microplate**

<b>Procedure</b>	<b>Time</b>
Prepare solutions.	2 min
Carefully remove 90% of media.	1 min
Pipette fixation solution into microplate.	1 min
Incubate with fixation solution.	10 min
Wash cells and add Reaction Solution to microplate.	2 min
Incubate with Reaction Solution.	60 min
Add Nuclei Dye Solution to microplate and incubate.	6 min
Remove Nuclei Dye Solution and add PBS.	3 min
<b>Total assay time</b>	<b>85 min</b>

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## 2. How to Use this Product

### 2.1 Before you begin

<b>Precautions</b>	<p>Take special precautions when working with biological research samples:</p> <ul style="list-style-type: none"><li>• Always wear protective gloves (powder-free).</li><li>• Handle all biological material as potentially infectious.</li><li>• Handling and disposal of potentially infectious biological material should be performed according to local safety guidelines.</li><li>• Spills should be immediately disinfected with an appropriate disinfectant solution to avoid spreading infection to, and contamination of, laboratory personnel and equipment.</li></ul> <p>⚠ The Label Solution contains cacodylate, toxic by inhalation and swallowed, and cobalt dichloride, which may cause cancer by inhalation. Avoid exposure and obtain special instructions before use. When using, do not eat, drink or smoke. After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell seek medical advice immediately (show label where possible). Collect supernatants from the labeling reactions in a tightly closed, nonbreakable container and indicate contents. Discard as regulated for toxic waste.</p>
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**Sample Material** Adherent cells

**Negative Control** Nontreated cells

**Positive Control** Cells treated with a concentration range of an apoptotic agent, *e.g.*, antimycin A.

**Cell Culture Conditions** Minimize intra- and inter-experimental variance by using cells that are regularly passaged, proliferating well (best when in a log-phase growth), and plated at a consistent density.

### 2.2 Experimental Protocol

**Preparation of Adherent Cells for the Assay** Plate cells in appropriate cell culture medium using a regular flat bottom 96-well microplate and at least 150  $\mu$ l per well. Cell confluence at time point of treatment should not be more than 70%. Induce apoptosis, by adding an apoptosis-inducing compound, such as antimycin A, in a dose-dependent manner, and then stain cells following the instructions below.

**Staining and Analyzing Adherent Cells** Ⓞ In the following procedure, it is important to perform all aspiration and wash steps carefully to ensure that cells remain attached to the microplate.

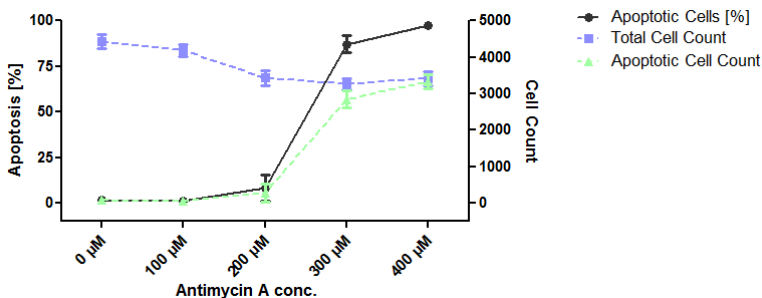
- ③ If cells adhere more weakly to standard microplate surfaces, Roche recommends using microplates that have been precoated with poly-d-lysine to ensure cells are not lost during washing steps. Cell cultures with apoptotic or necrotic cells may adhere more weakly to uncoated microplate surfaces.

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- ① Allow all reagents to equilibrate to +23 to +27°C.  
⚠ Protect vials from exposure to light throughout the procedure.
- 
- ② Prepare a fixation solution with 4% formaldehyde and 0.1% Triton-X 100 in purified water.
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- ③ Carefully remove approximately 90% of the media from cells (e.g., from 200  $\mu$ l supernatant remove 180  $\mu$ l).
- 
- ④ Add 180  $\mu$ l fixation solution to each well to fix the cells
- 
- ⑤ Incubate the microplate for 10 min at +23 to +27°C.
- 
- ⑥ While cells are incubating, prepare the Reaction Solution by combining 500  $\mu$ l Enzyme Solution (vial 2, yellow cap) and 4.5 ml Label Solution (vial 3, clear cap) in a separate tube, and vortex briefly  
③ The Reaction Solution should be prepared immediately before use and should not be stored.
- 
- ⑦ Remove fixation solution from the wells. Wash cells 1 $\times$  using 200  $\mu$ l PBS per well. Wash the cells by gently pipetting PBS into the wells, then immediately removing the PBS via pipetting. Ensure that the PBS is completely removed before continuing with Step ⑧.  
③ When removing fixation solution or PBS from the wells, ensure that the pipette tips are always placed in the corners of the wells to minimize disturbance of the adherent cell layers.
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- ⑧ Add 45  $\mu$ l of the Reaction Solution to each well.
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- ⑨ Incubate 60 min at +37°C.
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- ⑩ While cells are incubating, dilute 20  $\mu$ l Nuclei Dye (vial 1, blue cap) in 20 ml 1 $\times$  cell culture grade PBS. Store in the dark until use.
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- ⑪ Add 150  $\mu$ l of the Nuclei Dye mixture to each well.
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- ⑫ Incubate 5 min at +23 to +27°C in the dark.
- 
- ⑬ Carefully remove Nuclei Dye solution by pipetting. Ensure that all of the Nuclei Dye solution has been removed before proceeding with Step ⑭.  
③ When removing Nuclei Dye from the wells, ensure that the pipette tips are always placed in the corners of the wells to minimize disturbance of the adherent cell layers.
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- ⑭ Add 200  $\mu$ l PBS to each well.
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- ⑮ Analyze cells with a suitable fluorescence microscope using the appropriate excitation and emission filters, or an appropriate system for automated image acquisition and analysis or detection by flow cytometry.
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### 3. Results

Results in Figure 1 below, show NIH3T3 cells treated with various concentrations of antimycin A, and then processed as indicated below:

- ① NIH3T3 cells were plated in a standard 96-well microplate, using 5000 cells in 150  $\mu$ l media per well.
- ② Incubate the microplate for 24 h in a tissue culture incubator at +37°C, 5% CO<sub>2</sub>, 95% relative humidity.
- ③ Twenty-four hours after seeding the cells in the microplate, add 50  $\mu$ l antimycin A in different concentrations to each well.
- ④ In the example shown in Figure 1, antimycin A concentrations in the wells were 100  $\mu$ M, 200  $\mu$ M, 250  $\mu$ M 300  $\mu$ M, 400  $\mu$ M; the microplate also included control wells without antimycin A.
- ⑤ Incubate for 5 hrs and proceed as described in Chapter 2.2, "Staining and Analyzing Adherent Cells".



**Fig. 1:** Results of an analysis of antimycin A-induced apoptosis of NIH3T3 cells using the DNA Fragmentation Imaging Kit with the Cellavista System.

## 4. Troubleshooting

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
<b>Low labeling</b>	Fluorescence bleaching may occur upon prolonged exposure to bright light.	Keep samples in the dark after staining for later inspection.
	Permeabilization not effective so that reagents can not reach their target molecules.	<ul style="list-style-type: none"> <li>• Ensure that Triton-X 100 has been added to the fixation solution.</li> <li>• Increase incubation time for fixation.</li> </ul>
<b>High background</b>	Mycoplasma contamination	Use the Mycoplasma PCR ELISA Kit* to check for possible mycoplasma contamination.
<b>Nonspecific labeling</b>	Enzyme Solution concentration too high	Reduce concentration of Enzyme Solution by reducing the amount added to the Label Solution.
<b>Fewer labeled cells than expected</b>	Weakly adherent cells are more easily washed out of the plate during washing steps.	Use microplates precoated with poly-D-lysine to minimize cell loss when working with weakly adherent cells.
	Cells were washed too harshly resulting in loss of adherent cells.	Perform all aspiration and wash steps carefully to minimize the loss of cells.



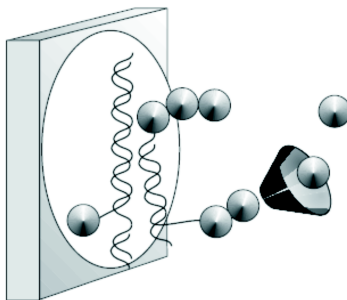
## 5. Additional Information on this Product

### 5.1 How this Product Works

Cleavage of genomic DNA during apoptosis yields double-stranded, low molecular weight DNA fragments (mono- and oligonucleosomes), as well as single strand breaks ("nicks") in high molecular weight DNA. These DNA strand breaks are identified by labeling free 3'-OH termini with modified nucleotides in an enzymatic reaction.

The TUNEL reaction preferentially labels DNA strand breaks generated during apoptosis. This allows discrimination of apoptosis from necrosis and from primary DNA strand breaks induced by cytostatic drugs or irradiation.

Using this kit, DNA strand breaks are labeled by Terminal deoxynucleotidyl transferase (TdT), which catalyzes polymerization of labeled nucleotides to free 3'-OH DNA ends in a template-independent manner (TUNEL-reaction). Fluorescein labels incorporated in nucleotide polymers are then detected and quantified by fluorescence microscopy.



**Fig. 2:** DNA of fixed cells labeled by the addition of fluorescein dUTP at strand breaks by terminal transferase.

Hoechst 33342 is a cell-permeable stain that binds to DNA and can be used for staining of nuclei of living or dead cells.

### 5.2 Quality Control

The kit is function tested using a cellular model (HeLa cells treated with anti-mycin A).

### 5.3 References

- 1 Whiteside, G., *et al.* (1998). An improved method for detection of apoptosis in tissue sections and cell culture, using the TUNEL technique combined with Hoechst stain. *Brain Research Protocols* **2**, 160-164.
- 2 Gavrieli, Y. *et al.* (1992). Identification of Programmed Cell Death In Situ via Specific Labeling of Nuclear DNA Fragmentation. *Journal of Cell Biology* **199**, 493-501.
- 3 Kim, KM, *et al.* (2001). Biophysical characterization of recombinant human Bcl-2 and its interactions with an inhibitory ligand, antimycin A. *Biochemistry* **40**, 4911-4922.

## 6. Supplementary Information

### 6.1 Conventions

**Text Conventions** To make information consistent and easy to understand, the following text conventions are used throughout this document:

Text Convention	Usage
Numbered stages labeled ①, ② <i>etc.</i>	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ①, ② <i>etc.</i>	Steps in a procedure that must be performed in the order listed.
Asterisk*	Denotes a product available from Roche Diagnostics.

### Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
ⓘ	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

### 6.2 Changes to Previous Version

- Editorial changes.

### 6.3 Ordering Information

Product	Pack Size	Cat. No.
Mycoplasma Detection		
Mycoplasma PCR ELISA	1 kit (96 reactions)	11 663 925 910

**6.4 Trademarks**

All other product names and trademarks are the property of their respective owners.

**6.5 Regulatory Disclaimer**

For life science research only. Not for use in diagnostic procedures

**6.6 Disclaimer of Licence**

For patent license limitations for individual products please refer to:  
[List of biochemical reagent products](#)

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