

1. Technical tips

1.1 Selected frequently asked questions (FAQs) about cell death assays

The questions below were chosen from those received by our Technical Services representatives. Wherever possible, the answers will direct you to pages and sections of this guide which can provide more information.

① Can I determine the number of apoptotic cells using the Cell Death Detection ELISA^{PLUS}?

A: No. The ELISA data is interpreted as a change in the level of death in an apoptotic population compared to an uninduced control population. It does not provide data on individual cells.

② What is the best way to get rid of non-specific (false-positive) background in the TUNEL (*In Situ* Cell Death Detection) kits?

A: The best approach to reducing background depends on the results you obtain with the controls:

- If cells incubated with fluorescein-dUTP but without terminal transferase are false positive, try washing the cells more thoroughly, reducing the concentration of fluorescein-dUTP, or using an alternative permeabilization procedure.
- If false positives are produced only in reactions which include both fluorescein-dUTP and terminal transferase, the best means of reducing false positives is a reduction in enzyme concentration or a change in permeabilization procedure.

Note: For further tips on obtaining the best results with the TUNEL method, see page 113 of this Appendix.

③ What types of sample can be assayed with the TUNEL method?

A: Tissue sections, adherent cell cultures, cytopins and cell smears have all been used with this assay (page 33, Section A 2.2.1). Note, however, that the sample material must be preserved with a cross-linking fixative (such as paraformaldehyde).

④ Why isn't substrate included in the TUNEL kits (*In Situ* Cell Death Detection Kits, AP or POD)?

A: These kits will work with a variety of common alkaline phosphatase or peroxidase substrates. Since many laboratories already have these substrates, and know how these substrates work in "their" system we decided to leave them out. In addition this gives the researcher the flexibility for secondary staining.

⑤ How long and at what temperature can I store my samples before analyzing them with the various kits that you offer?

A: Table 19 gives some general guidelines for sample storage. Note however that some samples may be more or less stable than others.

⑥ Is a special wash/stop buffer required for the TUNEL kits?

A: Our procedure does not require an equilibration buffer. Our wash buffer is PBS, a commonly used solution.

