

1.4 Technical tips on the use of the Apoptotic DNA Ladder Kit on tissue samples

The package insert for our Apoptotic DNA-Ladder Kit, Cat. No. 11 835 246 001, describes the purification of nucleic acids from whole blood and cultured cells. By following the modified procedure described here it is also possible to use tissue samples.

Preliminary Information

- **Weight of sample:** The tissue sample should weigh between 25 and 50 mg.
- **Additional required solutions:**
 - Lysis buffer: Prior to extraction of DNA, prepare a lysis buffer. 200 µl of this buffer are sufficient for one tissue sample. The lysis buffer consists of 4 M urea, 100 mM Tris, 20 mM NaCl and 200 mM EDTA, pH 7.4 (25°C).
 - Proteinase K solution: 20 mg/ml in 50 mM Tris-HCl (pH 8.0) and 1 mM CaCl₂.

Protocol for isolation of DNA from tissue samples

- 1 Add 200 µl lysis buffer and 40 µl proteinase K solution to 25–50 mg tissue, mix.
- 2 Incubate for 1 h at 55°C.
- 3 Add 200 µl binding buffer, mix.
- 4 Incubate for 10 min at 72°C.
- 5 Proceed with the addition of 100 µl isopropanol as described in the pack insert (3rd. step of section 5).

Note: Be aware, that apoptosis is a single cell event, and therefore in most tissues you will not find a sufficient number of apoptotic cells to produce a DNA ladder.

1.5 Technical tips on the Cell Proliferation ELISA Kits

How to interrupt the proliferation assay

The detection of BrdU-labeled DNA with the Cell Proliferation ELISAs does not take more than 1.5–3 hours. Nevertheless, the labeling period which may vary between 2 and 24 hours can get the scientist in time trouble. Our assay can be interrupted after the labeling process: After the removal of the culture medium, the protocol proceeds with the drying of the labeled cells using *e.g.*, a hair-dryer. The dry cells stay safe and sound up to one week when stored at 4°C in the microtiter plate before they are fixed and denatured according to the provided protocol.

A tip for measuring lymphocyte proliferation

To study the proliferation of lymphocytes, the cells are stimulated *e.g.*, with growth factors, cytokines or mitogens. The increase in cell numbers can (in special cases) lead to cluster formation of the lymphocytes: Cells from the same progenitor stick together and form aggregates in the culture plate. This effect may disturb the antibody recognition of the ELISA system and thereby result in an underestimation of response. To avoid signal variation: Carefully resuspend the cells after the BrdU-labeling period and before centrifugation for removing the culture medium. This will enable the equal accessibility of each cell for the antibody recognizing the BrdU-label.