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Product Information

Apo-TRACE® *In Vivo* Apoptosis Detection Kit

Catalog Number **CS1120**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Apoptosis, a form of programmed cell death, is a biological process important in normal development as well as pathological states. Many diseases are characterized by excessive apoptosis, among them AIDS, neurodegenerative disorders (e.g., Alzheimer's disease), ischemic infarcts in the brain, kidney, or heart, organ transplant rejection, graft versus host disease, and more.[1,2] In addition, most anticancer treatments (chemotherapy, radiotherapy, and immunotherapy) act by inducing apoptosis.[3]

Apo-TRACE® is a small, non-toxic, organic molecule, which is a member of the *ApoSense*® family of low molecular weight compounds used for imaging cell death *in vivo*. The *ApoSense* compounds respond to alterations in plasma membrane potential and phospholipid scrambling, which are hallmarks of apoptotic cells. Upon systemic administration, the compounds can mark apoptotic cells from the early stages of cell death.[4-6] The Apo-TRACE compound has inherent fluorescent properties (excitation at 328 nm and emission at 563 nm) and accumulates in the cytoplasm of apoptotic cells in the living body. Since most anticancer treatments act by inducing apoptosis, staining with Apo-TRACE can give a good indication for the efficiency of the treatment.

Components

The kit is sufficient for 25 injections into mice (20–27 g of animal weight).

Apo-TRACE solution (15 mg/ml) Catalog Number V7639	5 ml
CelLytic™ M - Mammalian Cell Lysis/Extraction Reagent Catalog Number C2978	40 ml

Reagents and Equipment Required but Not Provided

For Apo-TRACE staining

- Sterile syringes and needles
- Vessel for liquid nitrogen freezing
- Glass slides
- Cover glasses (Catalog Number C9802)
- Fluorescent microscope equipped with appropriate filters. The kit was developed using Olympus IX71 inverted microscope equipped with a Wide UV excitation with long pass emission filters (excitation 350/50 nm, emission 420 long pass) and Olympus BX51 equipped with a UMNIBA2 filter.
- Cryostat

For Apo-TRACE quantification

- Fluorimeter equipped with appropriate filters. Apo-TRACE was quantified using Synergy™ HT Fluorimeter equipped with filters for excitation at 360/40 nm, emission at 530/25 nm.
- Nunc-Immuno™ Microwell™ 96 well plates, black (Catalog Number P8741) or equivalent
- ULTRA-TURRAX® tissue homogenizer or pellet pestle with disposable tip (Catalog Number Z359971 or Z359947) or equivalent
- Centrifuge (capable of up to 20,000 × *g*).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit ships on dry ice and storage at –20 °C is recommended. After first thaw, store the Apo-TRACE solution in working aliquots at –20 °C. The CelLytic M reagent may be stored at room temperature.

Procedure

A. *In vivo* apoptotic cell staining

Note: The Apo-TRACE solution should be protected from light at all times.

Establish the specific *in vivo* model involving apoptosis processes. It is recommended to include a control animal (with no apoptosis inducer) to detect non-specific adsorption of Apo-TRACE.

1. Thaw the Apo-TRACE solution (Catalog number V7639). Warm at 37 °C before each experiment for at least 10 minutes. Vortex well and mix until homogenous.
2. Inject 200 µl (~110 mg/kg) of Apo-TRACE solution intravenously into the animal (e.g., into a mouse tail vein).
3. After 1–2 hours, sacrifice the animal.
4. Surgically dissect the desired organ or tissue, and place in a vessel suitable for freezing in liquid nitrogen.
5. Protect the specimen from light, by covering the vessel with aluminum foil.
6. Place the specimen in liquid nitrogen for 30–60 minutes.
7. Transfer the samples to –70 °C for at least 16 hours before preparation of sections.
   Note: Specimen fixation is not recommended.
8. Prepare a 5 µm cross-section of the frozen specimens on glass slides.
9. Analyze the sections using a fluorescent microscope equipped with the appropriate filters (see Reagent and Equipment Required but Not Provided).

B. Quantitative analysis of Apo-TRACE accumulation

Apo-TRACE uptake into apoptotic cells can be evaluated quantitatively by measuring the level of accumulation of the fluorescent dye within the cells of the organ or tissue. The detection of apoptosis by fluorescent methods depends on the labeling efficiency, which varies among cell/tissue types, cell number, or tissue weight, and the sensitivity of the detection instrument used. Therefore, the amount of Apo-TRACE needed for a particular tissue or research system should be determined empirically.

1. Excise the relevant organ or tissue from the animals labeled with Apo-TRACE.
2. Weigh the organ and place it in a tube containing CelLytic M reagent. The volume of CelLytic M reagent used should be 10 µl per mg of tissue.
3. Homogenize the organ with an ULTRA-TURRAX or a pellet pestle homogenizer.
4. Centrifuge the homogenate at 16,000–20,000 × *g* for 20 minutes at 2–8 °C to pellet the tissue debris.
5. Transfer the supernatants into a fresh tube protected from light. Place on ice.
6. Prepare standard solutions from the Apo-TRACE solution (15 mg/ml, Catalog number V7639) in CelLytic M reagent. Perform serial dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1,280.
7. Prepare an Apo-TRACE calibration curve by transferring a fixed volume (50-100 µl) of the Apo-TRACE standard solutions into separate wells of a black 96 well plate. Perform in triplicates.
8. Prepare the experimental sample by transferring the same fixed volume (50–100 µl) of the supernatant from step 5 into 2 separate wells of a black 96 well fluorescent plate. Avoid bubble formation.
9. Use the same fixed volume (50–100 µl) of CelLytic M solution as a blank.
10. Measure the fluorescence intensity of the Apo-TRACE in the wells using a fluorimeter equipped with the appropriate filters.
11. Draw a calibration curve of the average fluorescence of the standard solutions as a function of Apo-TRACE concentration. This calibration curve can be used to quantify the Apo-TRACE accumulated in the sample.
    Note: Most of the Apo-TRACE is extracted from the biological sample using this procedure. If required, repeat steps 2–5 for additional elution of the Apo-TRACE.

References

1. Schulze-Osthoff, K., et al., Apoptosis signaling by death receptors. *Eur. J. Biochem.*, **254**, 439-459 (1998).
2. Kam, P.C.A., and Ferch, N.I., Apoptosis: mechanisms and clinical implications. *Anaesthesia*, **55**, 1081-1093 (2000).
3. Labat-Moleur, F., et al., TUNEL apoptotic cell detection in tissue sections: critical evaluation and improvement. *J. Histochem. Cytochem.*, **46**, 327-334 (1998).
4. Reshef, A., et.al., Novel molecular imaging of cell death in experimental cerebral stroke. *Brain Res.*, **1144**, 156-164 (2007).
5. Aloya, R., et.al., Molecular imaging of cell death in vivo by a novel small molecule probe. *Apoptosis*, **11**, 2089-2101 (2006).
6. Damianovich, M., ApoSense: a novel technology for functional molecular imaging of cell death in models of acute renal tubular necrosis. *Eur. J. Nucl. Med. Mol. Imaging*, **33**, 281-91 (2006).

Appendix

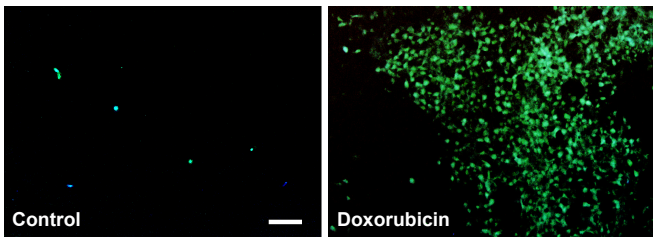
Example of an experimental model for apoptosis detection

The model is based on subcutaneous tumors treated with a chemotherapeutic drug that induces apoptosis.

1. Inject mice subcutaneously with 0.2 ml containing 5–10 $\times$ 10^4 C26 cells.
2. At day 16–18, or when tumors are already visible, inject mice intravenously (e.g., into the tail vein of the mouse) with the first dose of doxorubicin. Do not inject more than 0.2 ml (20 mg/kg).
3. 48 hours later inject the second dose of doxorubicin in the same manner.
4. 48 hours after the second doxorubicin injection, inject the animal with Apo-TRACE solution as indicated in the procedure.
5. 90–120 minutes later sacrifice the mice, excise the tumors, and prepare cryosections.
   Note: Visualization of Apo-TRACE staining in small tumors (5–6 mm diameter) is better than in larger tumors.

Figure 1.

Apo-TRACE detection of induced apoptosis in mice bearing C26 colon carcinoma



Balb/c mice (8–12 weeks old males) were injected subcutaneously with C26 colon carcinoma cells, treated with doxorubicin, and stained with Apo-TRACE as described in the Appendix. Tumors were excised and cryosections were prepared and mounted on glass slides. The slides were visualized using a fluorescence microscope. The extent of green fluorescence and intensity of Apo-TRACE accumulation in apoptotic foci represents the increased cell death induced by doxorubicin. The labeling in the control tumor reflects the basal apoptotic load in a non-treated tumor.

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ApoSense technology is covered by the following:
US patent application No. 10/433,668
US patent application No. 11/172,934
European patent application No. 01999555.4
Japanese patent application No. 547886/02
And related patent applications.

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