

# Induction of apoptosis in cells

Apoptosis may be induced in experimental systems through a variety of methods. In general, methods to induce apoptosis can be divided into two categories: biological induction and chemical induction.

## Biological induction of apoptosis

Activation of Fas or TNF receptors by their respective ligands, or by cross-linking with an agonist antibody, induces apoptosis of Fas- or TNF receptor-bearing cells. Here we describe a general protocol to induce apoptosis using an anti-Fas (anti-CD95) monoclonal antibody (mAb) in Jurkat cells. The mouse monoclonal [anti-Fas antibody \[UT-1\] \(ab11881\)](#) can be used for apoptosis induction.

1. Grow Jurkat cells in RPMI-1640 containing 10% fetal bovine serum (FBS) in a humidified 5% CO<sub>2</sub> incubator at 37°C.
2. Harvest exponentially growing cells at a concentration of 10<sup>5</sup> cells/mL by centrifugation at 300–350 x g for 5 min. Resuspend cells in fresh medium to a final concentration of 5 x 10<sup>5</sup> cells/mL.
3. Add anti-Fas (anti-CD95) mAb to the appropriate concentration (for ab11881, use a final concentration of 2 – 20 mg/mL). Incubate for 2–8 hr in a 37°C incubator. For a negative control, incubate untreated cells (without anti-Fas/CD95 antibody) under the same conditions.
4. Harvest the cells by centrifugation at 300–350 x g for 5 min.
5. Remove all medium and resuspend cells in PBS.
6. Repeat centrifugation step and resuspend cells in PBS to 1.5 x 10<sup>6</sup> cells/mL.
7. Proceed to detect apoptosis using your method of choice.

## Chemical induction of apoptosis

Apoptosis inducers act on several apoptosis-related proteins to promote apoptotic cell death. Depending on the agent selected and the concentrations used, apoptotic events can be detected between 8–72 h post-treatment. However, not all reagents will affect a particular cell line in the same way.

These are general guidelines for inducing cellular damage with chemical agents that will lead to apoptosis.

1. Set up your cells for treatment: inoculate adherent cells into 10 cm<sup>2</sup> tissue culture dishes or suspension cells into T75 flasks at a concentration of ~10<sup>6</sup> cells/mL. Prepare as many samples as necessary to ensure you can detect the induction of apoptosis at the relevant time. One dish/flask will be used as negative control for non-induced cells.
2. Add cellular damaging agents at the recommended concentrations to induce apoptosis.

For instance, the list below suggests final concentrations of cellular damaging agents that can be used for induction of apoptosis through p53-dependent G1 arrest:

Agent	Concentration
<a href="#">Doxorubicin hydrochloride (ab120629)</a>	0.2 µg/mL (25 µg/mL stock prepared in H <sub>2</sub> O)
<a href="#">Staurosporine (ab120056)</a>	1 µM (1 mM stock prepared in DMSO)
<a href="#">Etoposide (ab120227)</a>	1–10 µM (1 mM stock prepared in DMSO)
<a href="#">Camptothecin (ab120115)</a>	2–10 µM (1 mM stock prepared in DMSO)
<a href="#">Paclitaxel (ab120143)</a>	50–100 nM (stock prepared in DMSO)
<a href="#">Vinblastine (ab145649)</a>	60 nM (stock prepared in methanol)

Add appropriate volume of buffer or solvent to the negative control.

As a further control, inhibitors to the different pathways can be included:

Agent	Concentration
<a href="#">Z-VAD-FMK (ab120382)</a>	50 $\mu$ M (stock prepared in DMSO)

3. Harvest cells at different times (i.e. 8, 12, 16, 24, 48, 72 h) after the addition of the cellular damaging agent.
4. **For apoptosis detection:** Collect cells and detect apoptosis using your method of choice.
5. **For examination of apoptotic proteins:** Collect all cells. Prepare cell lysates for either western blot detection or immunoprecipitation using your method of choice. Always compare levels of apoptotic proteins with control treated cells to confirm induction.