Induction of Apoptosis

This protocol is provided only as a general guide. Researchers should standardize these assays for their own specific needs and should consult published literature.

Proteins such as p53, p21WAF1, Myc, Bcl-2, Bax, and Bak are involved in the regulation of apoptosis. The agents and doses listed in the table below can be used to induce apoptosis. Not every agent will induce apoptosis in every cell type; indeed, dexamethasone will actually stimulate growth of some cells. Depending on the agent selected and the concentrations used, maximal induction of a particular protein may occur within 8 to 72 hours post-treatment. We have found that not all proteins are affected by each of these reagents in a particular cell line. Even proteins involved in preventing apoptosis, such as Bcl-2, may induce apoptosis by these treatments (but with a different time course).

Inducers of Apoptosis:

Agent	Dose	Solvent for Stock Solution	Cat. No.
Actinomycin D	500 ng/ml	Methanol	114666
Aphidocolin	2 µg/ml	DMSO	178273
A23187	10 µM	DMSO	100105
Caffeine	16 mM	Boiling H_2O	205548
Camptothecin	4 µg/ml	DMSO	208925
Cycloheximide	100 µg/ml	H_2O	239764
Dexamethasone	1 µM	Ethanol	265005
Doxorubicin (Adriamycin)	0.2 µg/ml	H_2O	324380
5-Fluorouracil	25 µg/ml	DMSO, Hot H_2O	343922
Hydroxyurea	500 nM	H_2O	400046
Paclitaxel (TAXOL®)	100-580 nM	DMSO	580555
Staurosporine	500 nM	DMSO	569397
Thymidine	2 mM	PBS	6060
Vinblastine	60 nM	Methanol	677175

48-Hour Protocol for DNA Damage-Induced Apoptosis

The following protocol is based on p53-dependent G_1-arrest that occurs in response to DNA damage by chemical agents such as Doxorubicin, 5-Fluorouracil, Paclitaxel, and Vinblastine. A typical time course for p53 and p21^{WAF1} induction is 40 to 48 hours treatment with a DNAdamaging agent. Other proteins involved in apoptosis are also induced (although not all proteins involved in apoptosis will be induced by a particular agent in a given cell type). We recommend taking several time points (i.e. 24, 48, and 72 hours). Maximal induction of p21^{WAF1} requires wild type p53 activity. In the absence of wild type p53, p21^{WAF1} can also be induced by serum stimulation of G_1-arrested cells or by treatment with agents such as dexamethasone, albeit at significantly lower levels than that seen upon p53-dependent induction.

Day 1: Inoculate 2 or more 10 cm tissue culture dishes for adherent cells or T-75 flasks for non-adherent cells within approximately1 x 10^6 cells. One dish or flask will be used as a negative control for uninduced or basal level expression.

Day 2: Confirm that cells are growing by visual inspection of tissue culture dishes or by viable cell counts on non-adherent cells in T-75 flasks. Add DNA damaging agents at the indicated final concentration. Add appropriate volume of buffer or solvent (i.e. DMSO) to the uninduced control.

Day 3: Check cells to determine if cells have begun to die. Harvest cells if greater than 75% of the cells appear to have died.

Day 4: Harvest cells and prepare lysates for either Western blotting or immunoprecipitation as described in the accompanying protocols. For any agent used, a time course of induction can be performed by inoculating additional dishes or flasks and harvesting at various times after addition of the DNA damaging

agent. For the examination of apoptotic proteins dead cells should also be collected.

Day 5: Resolve proteins on SDS-PAGE. Visualize the protein of interest from total lysates by Western blotting using chemiluminescent detection as described in the accompanying protocols.

***Always compare levels of p53 or p21^{WAF1} from treated cells with those from untreated controls to confirm induction. For γ-irradiation treatment to induce p53 and p21^{WAF1}, see* El-Deiry, W. S., et al. 1994. *Cancer Res.* **54,** 1169 *or* Deng, C., et al. 1995. *Cell **82,** 675.*

- downloaded from: www.calbiochem.com -