

## CELL-BASED ASSAYS

### Apoptosis

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. The apoptotic process is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA<sup>1,2</sup>. Staining with FITC Annexin V is typically used in conjunction with a vital dye such as 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (7-AAD negative, FITC Annexin V positive).

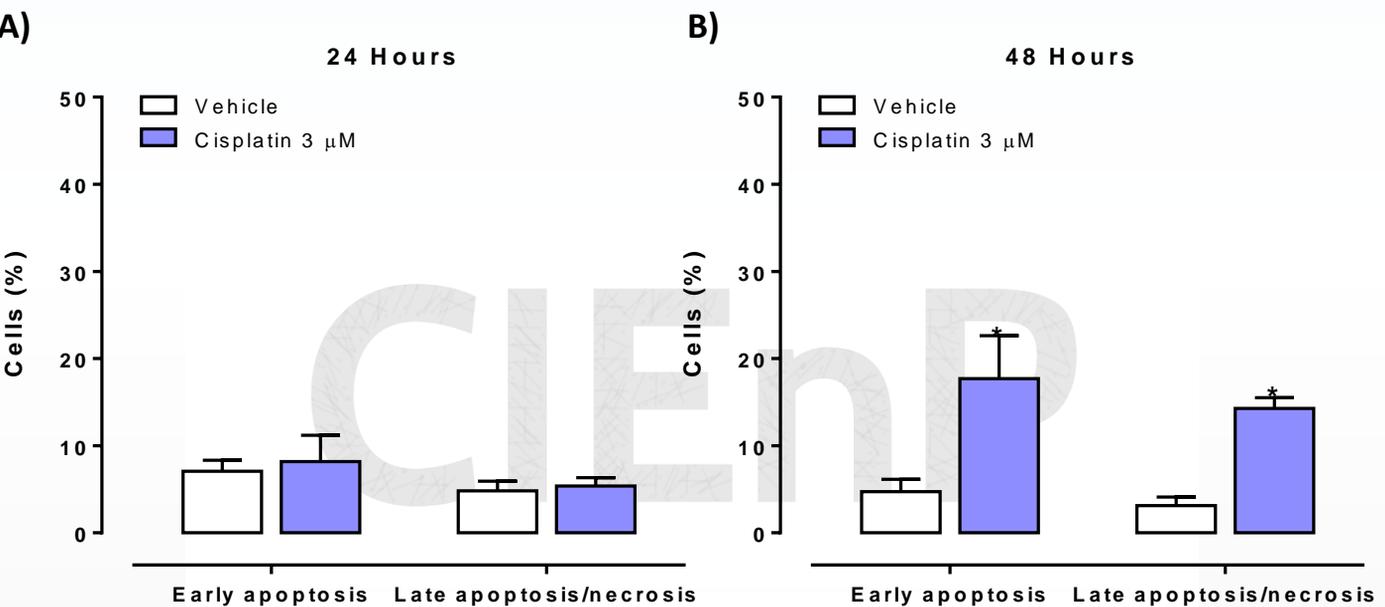
**Test system:** A375 (human melanoma) - ATCC®.

**Main Read-out:** Apoptosis.

**Experimental number:** Three wells per group in triplicate.

**Reference Item:** Cisplatin.

### Validation Data



**Figure:** Apoptosis assay carried out in human melanoma cells A375. The figure represents the apoptosis after cisplatin incubation through (A) 24 or (B) 48 hours and compared with control group (vehicle). Each column represents the mean ± SEM of 3 wells per group in triplicate. Statistical analyses used was *t-test*. \*P < 0.05 versus vehicle group.

To avoid bias and to allow reproducibility and reliability of all *in vitro* experiments we follow the “Guidance on Good Cell Culture Practice”<sup>3</sup>. All *in vitro* experiments were performed in triplicate wells for each condition and repeated at least three times.

#### References:

- Elmore S. Apoptosis: A Review of Programmed Cell Death. *Toxicol Pathol.* 2007; 35(4): 495–516.
- Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, Bao JK. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif.* 2012 Dec;45(6):487-98.
- Coecke S; Balls M; Bowe G; Davis J; Gstraunthaler G, Hartung T, Hay R, Merten OW, Price A, Schechtman L, Stacey G, Stokes W. Guidance on good cell culture practice: a report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim.* 2005, 33(3):261-87.