

CELL-BASED ASSAYS

Citotoxicity

Chemotherapy with cytotoxic drugs is the main treatment modality for certain types of cancer. In vitro cell-based assays are rapid, simple and sensitive and can be used to quantitatively measure the cytotoxic activity of several compounds through two colorimetric assays: MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and NRU (Neutral Red Uptake)^{1,2}.

Test system: A549 (human lung carcinoma) - ATCC®.
Experimental number: Three wells per group in triplicate.
Reference Item: Cisplatin.

Main Read-outs:
 Absorbance of MTT (570 nm) and NRU (540 nm);
 Inhibitory concentration (IC₅₀);
 Maximum inhibition.

Validation Data

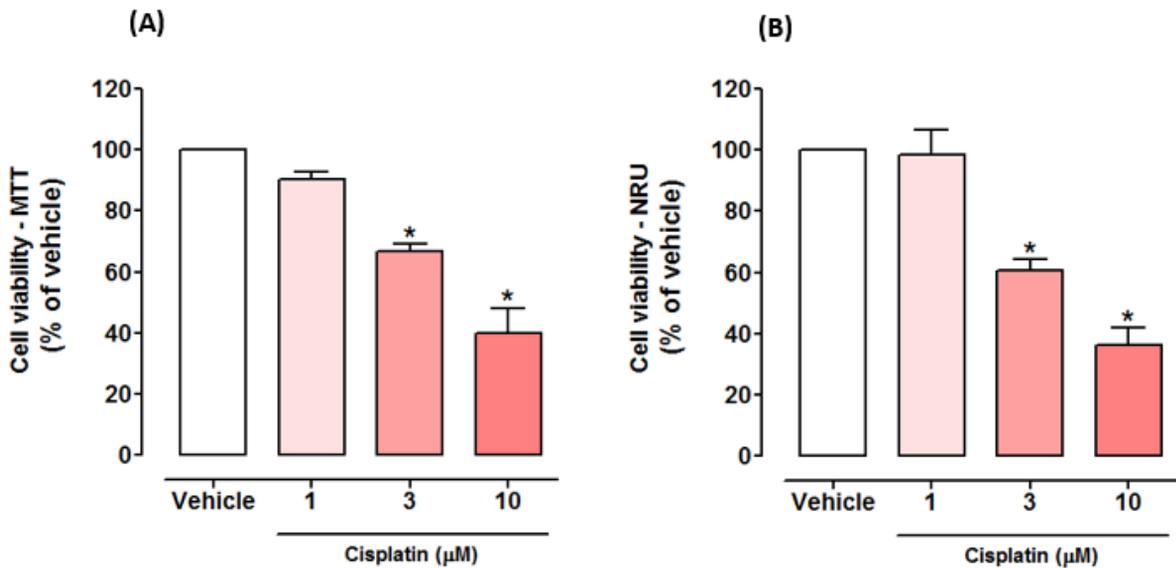


Figure: Citotoxicity assay of cisplatin through MTT and neutral red uptake (NRU). The figure represents the cell viability after cisplatin incubation through (A) MTT or (B) NRU assays in three concentrations when compared with control group (vehicle). Each column represents the mean ± SEM of 3 wells per group in triplicate. Statistical analyses used was one-way ANOVA with a post-hoc Dunnett's. *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”³. All in vitro experiments are performed in triplicate wells for each condition and repeated at least three times.

References:

¹Borenfreund E; Puerner JA. Toxicity determined in vitro morphological alterations and neutral red absorption. Toxicol Lett. 1985, 24(2-3): 119-24.
²Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec 16;65(1-2):55-63.
³Coecke 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.