

Application Note: Measure a Combination Index on Cell Death or Cell Growth.



Background.

Combination therapy represents an appealing approach to reduce the dose of drug treatment or to prevent the development of drug resistance. However, to determine the perfect combination strategy it is important to better characterize the mode of action of the drugs. We have developed a simple two-step assay that first, discriminates between a cytotoxic or an anti-proliferative effect for the drugs of interest and second, identifies the combination index of these drugs to yield the desired outcome.



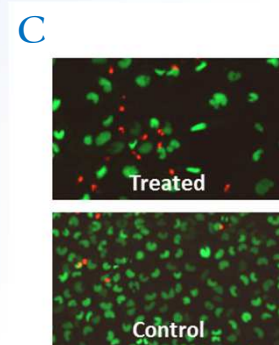
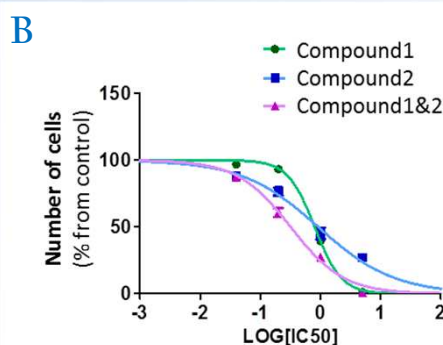
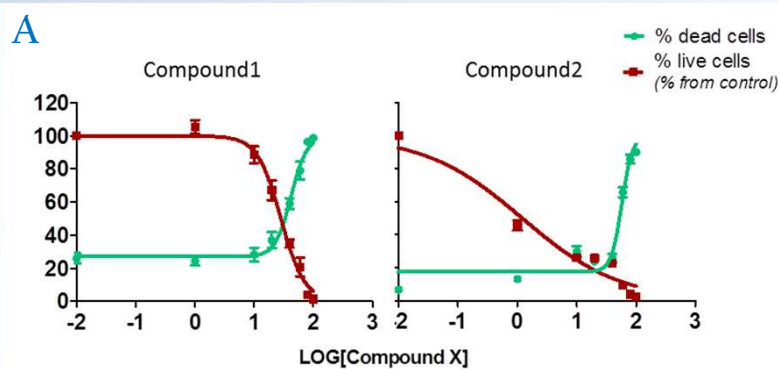
Method.

The Cell Cytotoxicity assay consists in the measure of two reliable end-points including the disruption of the cellular membrane and determination of the cell number (Figure C). This assay does not require washing steps, which can result in the disappearance of cell subpopulations. It can be performed in 384-wells plates and consequently only requires a small amount of compounds. Finally, the simplicity of this method dramatically limits the cost and time of each assay.



Applications.

The first step of the assay allows the discrimination between cytotoxic and antiproliferative effect for a given drug at a given concentration. For example, in Figure A, Compound 2 exerts an antiproliferative effect more pronounced than Compound 1 on a given cancer cell line. Indeed at 1 μM ("0" on the X axis), Compound 2 induces a drop of more than 50 % in cell number with only a small increase in cell death. This represents an essential information to determine if the combination index measurement (second step of the assay, Figure B) is more relevant based on the IC₅₀ (proliferation), EC₅₀ (cell death), or on both.



Measurement of the combination index of two drugs.

A. Determination of the IC₅₀ and EC₅₀ of two drugs on a cancer cell line. B. Determination of the combination index based on the IC₅₀ (proliferation) for two given drugs. C. Images correspond to cells stained with propidium iodide (dead cells) and Syto24 (total cells).

Questions? Please contact us: BMYSscreen@gmail.com