MTT ASSAY:

Principle: Rapid colorimetric assay based on the cleavage of the tetrazolium ring of MTT (3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide) by dehydrogenases in active mitochondria of living cells as an estimate of viable cell number.

1. Plate cells at approximately 104 cells/well in 96 well plates (10ml of 105 cells/ml/plate) and incubate for 24 hr at 37oC.
2. Aspirate old media, add 100ul of treatment media to all wells and incubate for a further 24 hr (treatment periods may be varied with test compound).
3. Dilute 100X stock MTT solution (50-100mg/mL) in media and add 100uL of working MTT solution to each well (10 mL working solution per plate) to achieve 0.5mg/mL. Incubate further for 4 hr (note: working MTT solution can either be added directly to treatments or the treatment media may be aspirated and replaced with working MTT solution).
4. After 4hr carefully aspirate MTT solution from each well and dissolve the formazan precipitate by adition of 100uL DMSO.
5. Read absorbance at 570nm in plate reader (correct for background using blank wells or use standards (i.e. control (no treatment) and blank (no cells)).