

### **Cellular Viability--XTT Assay Protocol**

- This assay is based on the conversion of the water-soluble XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reagent to an orange formazan product by actively respiring cells.
- Perform drug treatments @ 37°C.
  - Include a no-treatment control and a vehicle control for each drug.
  - Designate wells for a 'dead' control. These wells will be treated with a final concentration of 0.2% triton X-100 15 minutes before the end of the drug treatment.
  - Also designate wells that contain only media for 'no cell controls'.
- Before the end of the treatment, remove the electron coupling reagent and the XTT reagent from the -20°C and thaw in a 37°C water bath.
- Mix 100 µl of coupling reagent and 5 ml of XTT reagent to a reservoir and mix well.
  - Add 50 µl of the above XTT solution to each well using a multi-channel pipettor.
  - Return cells to the incubator for 4 hours (between 2 and 4 hours is sufficient).
- Read the absorbance at 490 and the 690 nm using a plate reader (1.0 sec/well).

### **Ordering information:**

- XTT kit, Roche #1465015.