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DATA SHEET

XTT Cell Viability Assay Kit

Catalog Number: 30007 (1000 assays)

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Description

XTT Cell Viability Assay Kit provides a simple method for determination of live cell number using standard microplate absorbance readers. Determination of live cell number is often used to assess rate of cell proliferation and to screen cytotoxic agents. XTT is a tetrazolium derivative. Similar to MTT, XTT measures cell viability based on the activity of mitochondria enzymes in live cells that reduce XTT and are inactivated shortly after cell death. Unlike the water-insoluble formazan produced from MTT, XTT is readily reduced to a highly water-soluble orange colored product [1,2], thus omitting the solubilization step required for the MTT assay. The amount of water-soluble product generated from XTT is proportional to the number of living cells in the sample and can be quantified by measuring absorbance at wavelength of 475 nm.

Kit Components

5 bottles (10mL each) of XTT Solution (sterile)
5 vials (50µL each) of Activation Reagent PMS (sterile)

Storage and Handling

Upon receipt the kit should be stored at 4°C and protected from light. Stored properly, the kit components should remain stable for at least 6 months.

Experimental Protocol

1. Plate cells into 96-well tissue culture plates. In general, cells should be seeded at densities between 5000 and 10,000 cells per well since they will reach optimal population densities within 48 to 72 hours.

2. Carry out your experiment by adding chemicals or biological agents. The final volume of tissue culture medium in each well should be 0.1mL, and the medium may contain up to 10% Fetal Bovine Serum.

3. For two 96-well plates, mix the contents of the XTT Solution vial and Activation Reagent vial. For one 96-well plate, mix 25µL Activation Reagent with 5mL XTT Solution to derive activated XTT solution and save the rest of XTT Solution and Activation Reagent for later use.
Note: If sediment is present in the XTT Solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

4. Add 25µL or 50µL of the activated XTT solution to each well and incubate the plate in an incubator for 2-24 hours (usually, 2-5 hours is sufficient).
Note: 50µL activated XTT solution generates a sharper increase of signal but reaches signal saturation at a lower cell number than 25µL activated XTT solution. 25µL activated XTT solution gives a broader dynamic range detection.

5. Shake the plate gently to evenly distribute the dye in the wells.

6. Measure the absorbance of the samples with a spectrophotometer (ELISA reader) at a wavelength of 450-500 nm. In order to measure reference absorbance (for non-specific readings), use a wavelength of 630-690 nm.

Note

1. Prepare the reaction mixture immediately prior to use.

2. Since the test is extremely sensitive, it is possible to use a low concentration of cells in the wells (approximately 5000 cells per well). Since there are cell types that show low metabolic activity, such as lymphocytes, kartinocytes and melanocytes, it is recommended to increase the concentration of cells to 2.5×10^5 cells per well, in order to obtain development of formazan color within a reasonable period of time.

3. Incubation time with the reaction mixture varies according to the type and concentration of the cells. Therefore, it is advisable to perform an initial test by reading the absorbance at various time lapses, i.e. after 4, 6, 8, and 12 hours using the same plate.

4. Prior to reading the absorbance with a spectrophotometer, the plate should be gently shaken in order to evenly distribute the dye in the wells.

5. If the volume of the media in each well is larger than 100µL, add a larger amount of reaction mixture by the same increment (i.e. 100µL reaction mixture to 200µL growth media).

References

1) *J. Infect. Dis*. **172**, 1153 (1995); 2) *J. Immunol. Methods* **159**, 81 (1993); 3) *J. Immunol. Methods* **147**, 153(1992); 4) *J. Immunol. Meth.* **142**, 257 (1991); 5) *J. Natl Cancer Inst.* **81**, 577 (1989); 6) *Cancer Res.* **48**, 4827 (1988).

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium