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Revised: October 29, 2012

Product Information

XTT Cell Viability Kit

Catalog Number: 30007
Unit Size: 1000 assays

Kit Contents

Component	Size
30007A: XTT Solution (sterile)	5 x 10 mL
Activation Reagent PMS (sterile)	5 x 50 uL

Storage and Handling

Upon receipt, store the kit at 4°C. Protect XTT solution from light. Kit components are stable for at least 6 months from date of receipt when stored as recommended.

Product 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.

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).

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 in order to reach optimal density within 48 to 72 hours.
   Note: For cell types that have low metabolic activity, such as lymphocytes, keratinocytes and melanocytes, it is recommended to increase the concentration of cells to $2.5x10^5$ cells per well, in order to obtain development of formazan color within a reasonable period of time.
2. Carry out desired cell treatments. The final volume of culture medium in each well should be 100 uL, and the medium may contain up to 10% serum.
3. Prepare working solution immediately before use. If sediment is present in the XTT Solution, heat the solution to 37°C and swirl gently until a clear solution is obtained. For each 96-well plate to be tested, mix 25 uL Activation Reagent with 5 mL XTT Solution to derive activated XTT solution. Volumes can be scaled as necessary (see note under step 4).
4. Add 25 uL or 50 uL of the activated XTT solution to the 100 uL of medium in each well.
   Note: 50 uL activated XTT solution generates a sharper increase of signal but reaches signal saturation at a lower cell number than 25 uL activated XTT solution. 25 uL activated XTT solution gives a broader dynamic range of detection. If the volume of medium in each well is larger than 100 uL, add a larger amount of reaction mixture by the same increment (i.e. 100 uL reaction mixture to 200 uL growth medium).
5. Incubate the plate in an incubator for 2-24 hours (usually, 2-5 hours is sufficient).
   Note: 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 points, i.e. after 4, 6, 8, and 12 hours using the same plate.
6. Shake the plate gently to evenly distribute the dye in the wells.
7. Measure the absorbance signal of the samples with a spectrophotometer at a wavelength of 450-500 nm. Measure background absorbance at a wavelength between 630-690. Subtract background absorbance from signal absorbance to obtain normalized absorbance values.

Related Products

Catalog number	Product
30006	MTT Cell Viability Assay Kit, 1000 assays
30025-1	Resazurin Cell Viability Assay Kit, 2500 assays
30026	Calcein AM Cell Viability Assay Kit, 1000 assays
30029	NucView™488 Caspase-3 Assay Kit for Live Cells, 100 assays

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