

WelCount™ Cell Proliferation Assay kit

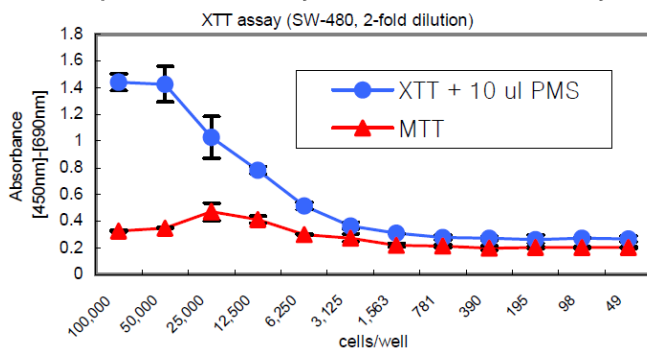
Catalog Number **TR 005-01**

Storage Temperature **-20°C/-70°C**

Product Description

WelCount™ Cell Proliferation Assay Kit is a product based on XTT(2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide), and measures the number of live cells through a spectrophotometer. The kit enables more convenient measurement of mitochondrial activity than a conventional MTT (Methylthiazolyldiphenyl-tetrazolium bromide) based products. The XTT system measures cell activity by detecting mitochondrial dehydrogenase, and the system yields highly accurate results through the simple process.

< Comparison of sensitivity between XTT and MTT assay >



Contents

XTT Reagent 5 ml X 4 ea
1% PMS Reagent 0.1 ml X 1ea

Storage/Stability

The reagents are stored at **-20°C/-70°C**.

Precautions

The reagents are filled to an amber container.

Protocols

- Because XTT and PMS reagent is light sensitive, it is recommended to minimize exposure to light.

1. **[Sample Preparation]** Adherent cells are seeded the day before the experiment and suspension cells are seeded immediately prior to the test.

*Note: Please attention to the contamination, because tetrazolium ring of XTT break by bacteria.

2. **[Thawing]** XTT and PMS reagents are thaw completely at 37°C water bath.

3. **[Preparation of working solution]** Working solution is produced at the rate of 20 ul PMS reagent per 1 ml XTT reagent and mix well.

*Note : Working solution is produced freshly on the day and it is recommend to use all.

4. **[Adding & Mixing]** Add the working solution of 20% volumes of culture media to each well and shake gently mix. For example, if the culture media volume is 200 ul, add 40 ul working solution.

5. **[Incubation]** Incubate at 37°C CO₂ incubator for 2 ~ 4 hours.

*Note: Please compare to measure at 2 hour and 4 hour, because cell line and number may differ.

6. **[Tapping]** At the end of reaction, the formed XTT formazan is mix well by tapping.

7. **[Reading]** Measure the absorbance using the plate reader.

*Note: The results = value measured at 450 nm – value at 690 nm

References

Tada, H, et al, (1986), *J Immunol Meth* 93, 157-165
 Scudiero, PA, et al, (1988), *Cancer Res* 48, 4827-4833
 Weislow, OS, et al, (1989), *J Natl Cancer Inst* 81, 577-586
 Hansen, MB, et al, (1989), *J Immunol Meth* 119, 203-210
 Roehm, NW, et al, (1991), *J Immunol Meth* 142, 257-265
 Jost, LM, et al, (1992), *J Immunol Meth* 147, 153-165
 Goodwin, CJ, et al, (1995), *J Immunol Meth* 179, 95-103
 Kairo, SK , et al, (1999), *Vaccine* 17, 2423-2428

Troubleshooting Guide

Problem	Check point
The results appear strangely	XTT and PMS reagent is not stored correctly (e.g., keep 4°C) Freezing and thawing cycle repeats
	Long-term use of the working solution (XTT and PMS mixture)
	Using the contaminated cells or too many cells
	The mixing ratio of the working solution is incorrect (XTT:PMS=1,000:20)
	The working solution is not added properly (20 ul working solution/ 100 ul culture media)