



Cell Proliferation/Toxicity Assay Reagents and Protocols

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We offer four different reagents for the measurement of cell proliferation and cytotoxicity: an ATP/Luciferase Assay (TCI Product No. **A3519** or **A3495**) (chemiluminescence-based), MTT (TCI Product No. **M3353**) (absorbance-based), WST-8 (TCI Product No. **W0023**) (also absorbance-based), and resazurin (TCI Product No. **R0195**) (fluorescence-based). Each reagent differs in measurement format (absorbance, fluorescence, chemiluminescence), allowing you to select the optimal reagent for your needs based on cost and sensitivity.

[Sample (Cell) Preparation] (Common to all assays)

Keeping the requirements of your specific experiment in mind, seed cells at an appropriate density (*1) into the appropriate number of wells (*2) of a 96-well microplate.

*1

"Appropriate cell density" refers to a density at which the cells do not become confluent before measurement.

*2

When measuring "proliferation", samples are prepared for two time points: one to be measured on the day the experiment starts, and the other several days later. Assays are performed at these time points, and the ratio of proliferation is calculated using the following formula. Note that the blank value refers to measurement of a well into which no cells were seeded.

$$\text{Proliferation (\%)} = \frac{(\text{Measurement value}_{\text{Day } n} - \text{Blank value}_{\text{Day } n})}{(\text{Measurement value}_{\text{Day } 0} - \text{Blank value}_{\text{Day } 0})} \times 100$$

When measuring "cytotoxicity", two samples are prepared: a treatment group and a control group. After a few days of treatment, assays are performed on samples from both groups, and cytotoxicity is calculated using the following formula. Note that the blank value refers to measurement of a well into which no cells were seeded.

$$\text{Cytotoxicity (\%)} = \left(1 - \frac{(\text{Measurement value}_{\text{Treatment group}} - \text{Blank value})}{(\text{Measurement value}_{\text{Control group}} - \text{Blank value})} \right) \times 100$$

[ATP-Luciferase assay system (chemiluminescence-based)]

Reagents

- [ATP-Luciferase Cell Viability Assay Solution](#) (TCI Product No. **A3495**)
- [ATP-Luciferase Cell Viability Assay Solution \(1.0mL×10\)](#) (TCI Product No. **A3519**)

1. Thaw an appropriate amount of assay solution (100 µL per well for a 96-well plate) on ice for 10-30 minutes.
2. Add the assay solution to the pre-prepared cells without removing the medium, using an volume equal

- to that of the medium (typically 100 μ L). Add the same amount of medium to blank wells in advance.
3. Incubate at room temperature for 10-15 minutes with gentle shaking.
 4. After incubation, measure chemiluminescence using a microplate reader.
 5. Calculate proliferation or cytotoxicity based on the formula above.

[MTT assay system (absorbance-based)]

Reagent

- [MTT Solution \[for Cell proliferation assay\] \(1mL×5\)](#) (TCI Product No. **M3353**)

1. Thaw an appropriate amount of assay solution (10 μ L per well for a 96-well plate) on ice for 10-30 minutes.
2. Add 10 μ L of assay solution to each well to be measured.
3. Incubate at 37 °C in a 5% CO₂ incubator for 2–4 hours.*
4. Remove the medium, taking care not to aspirate cells.
5. Add 100 μ L of DMSO to dissolve the purple reaction product.
6. Measure the absorbance at 570 nm using a microplate reader and calculate proliferation or cytotoxicity based on the formula above.

* The optimal incubation time varies depending on factors such as the specific cell line being used; please take appropriate considerations.

[WST-8 assay system (absorbance-based)]

Reagent

- [WST-8 Reagent \[for Cell Proliferation Assay\]](#) (TCI Product No. **W0023**)

1. Add 10 μ L of assay reagent to each well to be measured.
2. Incubate at 37 °C in a 5% CO₂ incubator for 1-4 hours.*
3. Measure the absorbance at 460 nm using a microplate reader and calculate proliferation or cytotoxicity based on the formula above.

* The optimal incubation time varies depending on factors such as the specific cell line being used; please take appropriate considerations.

[Resazurin assay system (fluorescence-based)]

Reagent

- [Resazurin \(Ready-to-use solution\) \[for Cell proliferation assay\]](#) (TCI Product No. **R0195**)

1. Add 10 μ L of assay reagent to each well to be measured.
2. Incubate at 37 °C in a 5% CO₂ incubator for 2-24 hours.*
3. Measure the fluorescence intensity (excitation: 540-570 nm / emission: 590 nm) using a microplate reader and calculate proliferation or cytotoxicity based on the formula above.

* The optimal incubation time varies depending on factors such as the specific cell line being used; please take appropriate considerations.