Cytotoxicity Assay Kit

ARG83600 Cytotoxicity Assay Kit is a detection kit for the quantification and monitoring of cell viability and growth.

Catalog number: ARG83560

Package: 96 assay

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PRINCIPLE OF THE ASSAY

The ARG83600 Cytotoxicity Assay Kit provide both colorimetric and fluorometric formats for measuring cell viability. The kit includes MTT reagent, Calcein AM, and Ethidium Homodimer, along with Detergent and Lysis Buffer for extracting these reagents from cell samples.

This kit can compatible with light and fluorescence microscopes, colorimetric and fluorometric multiwell plate scanners, flow cytometers, and other detection systems. Live cells are detected by MTT and Calcein AM, while dead cells are identified with EthD-1. Cell viability and cytotoxicity are assessed using both colorimetric and fluorometric methods.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store **500X Calcein AM** and **500X EthD-1** at -20°C, all other components store at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
100X Saponin	100 µl	4°C
500X Calcein AM	50 µl	-20°C
500X EthD-1	50 µl	-20°C
Detergent Solution	10 ml	4°C
MTT	1 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- 96 black wall microplate
- Microplate reader
- Pipettes and pipette tips
- Cell and Cell culture medium
- Deionized or distilled water
- Microscope

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store **500X Calcein AM** and **500X EthD-1** at -20°C, all other components store at 4°C. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

Calcein AM / EthD-1 Solution- Prepare this reagent immediately prior to use and use it within 20 min after preparation. Mix the 500X Calcein AM, 500X EthD-1, and culture medium at a **1:1:498** ratio.

1X Saponin Solution- Dilute the **100X Saponin solution** into culture medium to yield **1X Saponin Solution**. The 1X Saponin Solution is stable for up to 1 week at 2-8°C. Mix well before use.

ASSAY PROCEDURE

Cell culture

1. Seed **10,000 – 50,000 cells** to 96-well or **50,000 - 100,000 cells** to 24-well. Culture the cells 12-24 hours at 37°C at 5% CO_2.
   - The time and culture conditions depend on the cell line used and may need to be adjusted by the user.
2. Wash each well three times with cell culture medium to remove loosely attached and dead cells.
   - 1x Saponin solution can use to induce cell death as control.

MTT Colorimetric Detection

1. Add **100 µl** of the **Cell culture medium** into each 96-well, **250 µl** for 24-well.
2. Add **10 µl** of the **MTT** into each 96-well, **25 µl** for 24-well.
3. Incubate for **2-4 hours** or **overnight** at **37°C**.
   - Monitor the cells with an inverted microscope for the presence of a purple precipitate.
4. After precipitate is visible and clearly, add **100 µl** of the **Detergent Solution** into each 96-well, **250 µl** for 24-well.
5. Incubate for **2-4 hours** or **overnight** at **RT** in the dark.
6. Read the OD with a microplate reader at 450 nm.
   - If the values appear to be low, incubate the plate longer in the dark.

Calcein AM / EthD-1 Detection

1. Add **100 µl** of the **Calcein AM / EthD-1 Solution** into each 96-well, **400 µl** for 24-well.
2. Incubate for **30 minutes** at **37°C**.
3. Aspirate each well and wash with **Cell culture medium**, repeating the process for a total 2 time washes.
4. After the last wash, add enough medium to cover the cells.
5. Monitor the cells microscopically for the presence of the green **Calcein AM** (Ex/Em: 485 / 515 nm) or red **EthD-1** (Ex/Em: 525 / 590 nm) fluorescence. The fluorescence can be quantitatively measured with a fluorescence microplate reader.