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Calcein/PI Cell Viability/Cytotoxicity Assay Kit

Cat. No.	Product Name	Pack Size
C2015S	Calcein/PI Cell Viability/Cytotoxicity Assay Kit	100T
C2015M	Calcein/PI Cell Viability/Cytotoxicity Assay Kit	500T
C2015L	Calcein/PI Cell Viability/Cytotoxicity Assay Kit	2500T

Description:

- The Calcein/PI Cell Viability/Cytotoxicity Assay Kit provides a convenient method for detecting cell viability and cytotoxicity of animal cells based on Calcein-AM (Calcein AM) and Propidium iodide (PI) double fluorescence staining of viable and dead cells simultaneously.
- This kit requires 30 minutes approximately for fluorescence staining. Stained cells can be examined by fluorescence microscopy, flow cytometry, or fluorescence microplate reader for quantification analysis.
- Calcein AM is a non-fluorescent, hydrophobic compound that easily permeates intact, live cells. The hydrolysis of Calcein AM by intracellular esterases produces calcein, a hydrophilic and strongly fluorescent compound, which is well-retained in the cell cytoplasm and stain viable cells with strong green fluorescence. Dead cells can not be labeled with fluorescent calcein because dead cells have no or very low esterase activity. However, PI is a cell membrane impermeable dye and only stains dead cells with compromised cell membrane integrity, emitting bright red fluorescence when binding to DNA in nuclei of dead cells. Therefore, Calcein AM can be used in combination with PI for simultaneous detection of cell viability and cytotoxicity.
- Calcein generated from esterase in viable cells emits a strong green fluorescence with an excitation and emission maximum at 494nm and 517nm, respectively, while PI once bound to DNA has a maximum emission wavelength at 617nm when excited at 535nm. Please see Figure 1 for the excitation and emission spectra of Calcein and PI-DNA complex.

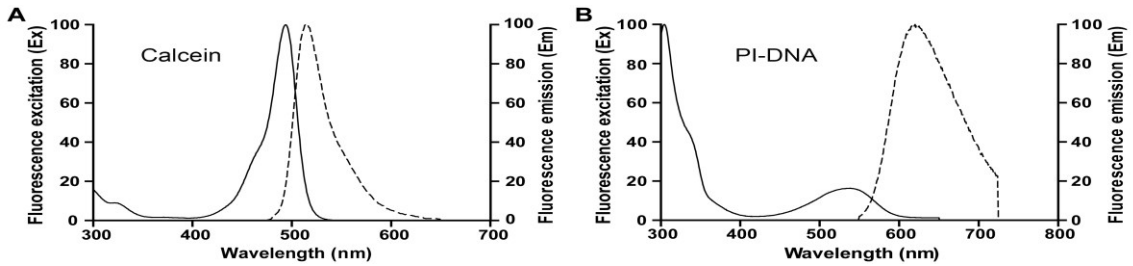

Figure 1. Excitation and emission spectra of Calcein and PI-DNA complex.

- Calcein AM stains live cells with green fluorescence, while PI stains dead cells with red fluorescence. Please see Figure 2 for the staining result of L929 cells with the Calcein/PI Cell Viability/Cytotoxicity Assay Kit (Beyotime, C2015).

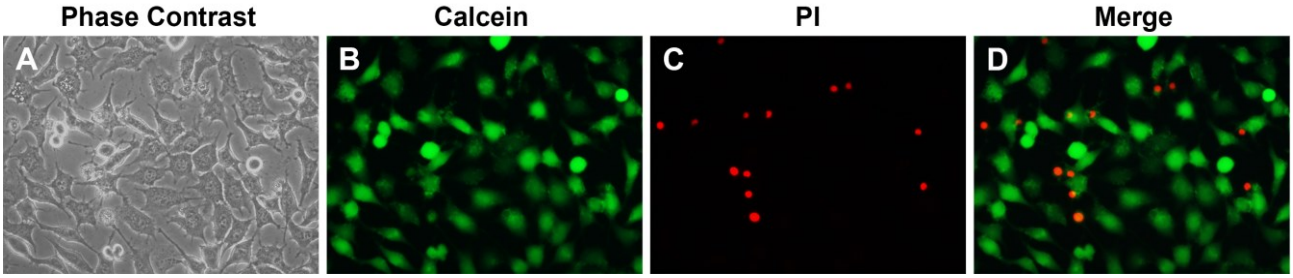

Figure 2. L-929 cells were stained with the Calcein/PI Cell Viability/Cytotoxicity Assay Kit (Beyotime, C2015) and examined by fluorescence microscopy. TSZ is a programmed cell necrosis inducer (Beyotime, C1058,) composed of TNFα, SM-164 and Z-VAD-FMKA. L-929 cells were pretreated with TSZ for 3 hours before being stained with this product. A: L-929 cells observed in the bright field. B: L-929 cells observed in the green fluorescence channel. Live cells were stained by green fluorescent calcein. C: L-929 cells observed in the red fluorescence channel. Dead cells were stained by the red fluorescent PI. D: Merge image of green and red channels.

- The product is applicable for most mammalian cells. It has been reported that Calcein AM can also be applied to plant cells such as root border-like cells from Arabidopsis, yeasts such as P*ichia anomala* and *Saccharomyces cerevisiae*, and parasites such as Leishmania. Due to the presence of some special components in cell membrane of some parasites, Calcein AM cannot stain their viable cells unless cells are at the early stage of apoptosis. Calcein AM is not suitable for staining fungi and bacteria because they have cell walls that prevent Calcein AM from entering cells. This kit is more sensitive and convenient than trypan blue staining method that has similar functions.
- Calcein AM and PI included in this kit are 1000X concentrated solutions, which is very convenient to use. The formula of both solutions are optimized to ensure their compatibility with most cells. However, the concentration of these two dyes should be optimized for different types of cells and for different experimental conditions to obtain good staining result, with the dilution factor ranging from 1:500 to 1:2000. Moreover, the Assay Buffer contained in this kit is better than PBS or HBSS in providing nutrients to cells to maintain their normal state over time during the staining process.
- When 0.1ml of reaction volume is used per well of a 96-well plate, reagents provided in C2015S, C2015M and C2015L are sufficient for 100, 500 and 2500 tests, respectively.

Packing List:

Item	Component	Quantity
C2015S-1	Calcein AM (1000X)	12μl
C2015S-2	PI (1000X)	12μl
C2015S-3	Testing buffer	12ml
Manual	—	1 copy

Item	Component	Quantity
C2015M-1	Calcein AM (1000X)	55μl
C2015M-2	PI (1000X)	55μl
C2015M-3	Testing buffer	55ml
Manual	—	1 copy

Item	Component	Quantity
C2015L-1	Calcein AM (1000X)	260μl
C2015L-2	PI (1000X)	260μl
C2015L-3	Testing buffer	260ml
Manual	—	1 copy

Storage Conditions:

Store kit at -20ºC for up to 1 year. Calcein AM (1000X) and PI (1000X) should be protected from light.

Precautions:

- Fluorescence quenches over time. Please be away from light while handling fluorescent dyes or stained samples to slow down the quenching of fluorescence.
- Calcein AM (1000X) decomposes easily in humid environment. Aliquot upon receipt, seal tightly and store at -20ºC.
- Calcein AM is unstable in aqueous solution. Prepare the caclcein AM working solution freshly prior to use. Do not freeze.
- Serum and phenol red in culture medium may influence the assay to some extent by increasing the fluorescent background. We recommend washing cells properly before the addition of calcein AM working solution to cells.
- This kit is for R&D only. Not for drug, household, or other uses.
- For your safety and health, please wear lab coat and disposable gloves during the operation.

Instructions for Use:

1. Preparation of Calcein AM/PI working solution

For 96-well cell culture plate, use 100μl of calcein AM/PI working solution per well. Based on the number of tests, prepare appropriate amount of calcein AM/PI working solution as indicated in the following table and mix thoroughly.

	10 tests	100 tests	1000 tests
Calcein AM (1000X)	1μl	10μl	100μl
PI (1000X)	1μl	10μl	100μl
Assay Buffer	1ml	10ml	100ml
calcein AM/PI woring solution	1ml	10ml	100ml

Note 1: *To obtain a desirable staining result, the dilution factor of Calcein AM (1000X) and PI (1000X) can be optimized from 500 to 2000 dilutions depending on the type of cells and the staining result.*

Note 2: *The calcein AM/PI working solution should be prepared freshly. Discard the rest after use.*

Note 3: *Calcein AM (1000X) may also be diluted with other suitable buffers such as serum-free culture medium, HBSS (Beyotime, C0218) or PBS (Beyotime, C0221A/C0221D). The Assay Buffer contained in this kit is usually more effective than PBS or HBSS in maintaining a normal state of cells and providing some nutrients to cells for a period of time.*

2. Cell viability/cytotoxicity assay with fluorescence microscopy:

a. **Cell growth.** Culture cells in multi-well plate, cell culture dish, or on a cell climbing sheet. Treat cells with desired method.

b. **Washing.** For adherent cells, remove the culture medium and wash cells once with PBS. For suspension cells, centrifuge at 250-1000×g for 5 min at room temperature, remove the supernatant and wash cells once with PBS. Phenol red or serum contained in culture medium has certain interference with the staining, which must be removed completely. We recommend using a vacuum pump to remove the culture medium and PBS. PBS washing can be omitted if culture medium can be removed thoroughly.

c. **Staining**. Add appropriate amount of calcein AM/PI working solution (100μl per well of a 96-well plate, 250μl per well of 24-well plate, 500μl per well of 12-well plate, or 1ml per well of 6-well plate). Incubate at 37ºC for 30min in the dark.

Note 1: *The final concentration of the Calcein AM/PI needs to be empirically determined for different cell types and/or experimental conditions.*

Note 2: *The optimal incubation time is different for different cell types, which can be optimized based on the staining results.*

d. **Examination** by fluorescence microscope (Calcein, Ex/Em=494/517nm; PI-DNA, Ex/Em=535/617nm). If necessary, cells could be counter-stained with other fluorescence, such as Hoechst 33342 (Beyotime, C1027/C1028/C1029) to stain nucleus.

Note: *The staining process and stained samples should be away from light as much as possible.*

3. Cell viability/cytotoxicity assay with flow cytometry

a. Cell preparation. Resuspend adherent cells by trypsin digestion and wash cells once with PBS. For suspension cells, centrifuge at 250-1000×g for 5min, remove the supernatant and wash cells once with PBS. We recommend to use 1×10^6 cells for each sample.

b. Staining. Add 1ml of calcein AM/PI working solution to cell pellet obtained above and resuspend cells gently and thoroughly. Incubate at 37ºC for 30min in the dark.

Note: *It is necessary to prepare a negative control cell sample resuspended in Assay Buffer without calcein AM/PI dyes. Meanwhile, two additional cell samples stained with Calcein AM or PI, respectively, should be prepared for compensatory adjustment of single dye.*

c. Examination by flow cytometry. After staining, the cells can be examined by flow cytometry directly. Stained cells could also be precipitated by centrifuge at 250-1000×g for 5min, and then re-suspended with 0.5ml of buffer after removing the supernatant, followed by analysis with a flow cytometry (Calcein, Ex/Em = 494/517nm; PI, Ex/Em = 535/ 617nm). If necessary, cells could be counter-stained with other fluorescence, such as Hoechst 33342 (Beyotime, C1027/C1028/C1029) to stain nucleus.

Note 1: *Protect from light during the staining and keep stained samples on ice in the dark until examination. Examination of cells should be completed within 1 hour.*

Note 2: *Use unstained negative control sample to set up the parameters of flow cytometry.*

Note 3: *When gating cells, be careful not to enclose cell debris. Calcein or PI single staining sample should be used to perform compensatory adjustment. Two relatively independent cell populations should be obtained when examined by flow cytometry assay: live cell population with green fluorescence and dead cell population with red fluorescence.*

Note 4: *Flow cytometry is very sensitive. The concentration of fluorescent probe used for flow cytometry is usually lower than that for fluorescence microscopy. The dilution factor of Calcein AM or PI should be adjusted appropriately.*

4. Cell viability/cytotoxicity assay with fluorescence microplate reader

a. **Cell growth.** Culture cells in a 96-well opaque plate, such as BeyoGold™ 96-Well Black Opaque Plate (FCP966). The number of cells per well needs to be controlled within 100-10,000. We recommend to start with 2000-5000 cells per well. Treat cells with desired method.

b. **Washing.** For adherent cells, remove the culture medium and wash cells once with PBS. For suspension cells, centrifuge at 250-

1000×g for 5 min at room temperature, remove the supernatant and wash cells once with PBS. Phenol red or serum contained in culture medium has certain interference with the staining, which must be removed completely. We recommend to use a vacuum pump to remove the culture medium and PBS. PBS washing can be omitted if culture medium can be removed thoroughly.

c. **Staining.** Add 100μl of Calcein/PI working solution per well and incubate at 37ºC in the dark for 30 minutes. The optimal incubation time varies for different type of cells, which can be adjusted and optimized based on the staining results.

d. **Examination.** After incubation, measure the fluorescence with a fluorescence microplate reader (Calcein, Ex/Em=494/517nm; PI, Ex/Em=535/617nm). The changes of live cell and dead cell amount can be obtained based on the RFU (Relative fluorescence values) of calcein and PI fluorescent dyes.

5. Determine the ratio of dead to live cells by using a fluorescence microplate reader.

The ratio of dead to live cell can be determined by setting up appropriate controls as follows:

a. Culture and treat cells as described in Part 4.

b. Besides the Calcein AM/PI working solution, Calcein AM working solution and PI working solution should be prepared respectively, with the same dilution factors as that used for preparation of calcein AM/PI working solution.

c. Setup samples an controls as follows:

Labeling	Group Name	Working Solution	Excitation Maximum	Emission Maximum	Result Name
(1)	Sample	Calcein AM/PI	494nm	517nm	$F(517)_{sam}$
(2)	Sample	Calcein AM/PI	535nm	617nm	$F(617)_{sam}$
(3)	Live cell group	PI	494nm	517nm	$F(517)_{min}$
(4)	Live cell group	Calcein AM	494nm	517nm	$F(517)_{max}$
(5)	Dead cell group	PI	535nm	617nm	$F(617)_{max}$
(6)	Dead cell group	Calcein AM	535nm	617nm	$F(617)_{min}$
(7)	Cell-free group	Calcein AM/PI	494nm	517nm	$F(517)_0$
(8)	Cell-free group	Calcein AM/PI	535nm	617nm	$F(617)_0$

Note 1: *Live Cell group are cells without drug treament; Dead cells are obtained by treating cells with 0.1-0.5% digitalis saponin (Beyotime, ST1272) or 0.1% aponin for 10 minutes, or 70% ethanol for 30 minutes.*

Note 2: *Except different cells in different groups, all other conditions are exactly the same, such as cell number, dye concentration, incubation time and incubation temperature etc.*

d. Stain cells and measure the RFU as described in Part 4.

e. Calculate the ratio of dead cells to live cells according to the following formula:

$$\%\ \text{Live Cells} = \frac{F(517)_{sam} - F(517)_{min}}{F(517)_{max} - F(517)_{min}} \times 100\%$$

$$\%\ \text{Dead Cells} = \frac{F(617)_{sam} - F(617)_{min}}{F(617)_{max} - F(617)_{min}} \times 100\%$$

Note 1: *The $F(517)$ and $F(617)$ values in the formula are obtained by subtracting the $F(517)_0$ and $F(617)_0$ from the original RFUs of all samples.*

Note 2: *To determine the exact cell number of live and dead cells, a standard curve can be prepared with different numbers of live and dead cells on abscissa and their corresponding RFU at 517nm and 617nm on ordinate, respectively. There is a linear relationship between live cell number and RFU at 517nm, or between dead cell number and the RFU at 617nm, by which the exact cell number of live and dead cells in a sample can be determined based on their RFU values.*

Related Products:

Cat. No.	Product Name	Pack Size
C0003	Apoptosis, Hoechst Staining Kit	100T
C0005	Apoptosis Inducers Kit	200T
C0006S	Apoptosis Inducer Kit (TNF-α+SM-164)	100T
C0007	Apoptosis, DNA Ladder Extraction Kit	50T
C0008	Apoptosis, DNA Ladder Extraction Kit with Spin Column	50T
C0011	Trypan Blue Staining Cell Viability Assay Kit	100T
C1052	Cell Cycle and Apoptosis Analysis Kit	50T
C1056	Apoptosis and Necrosis Assay Kit	100T

C1058S/M	Necroptosis Inducer Kit with TSZ	100/500T
C1062S/M/L	Annexin V-FITC Apoptosis Detection Kit	20/50/100T
C1065S/M/L	Annexin V-PE Apoptosis Detection Kit	20/50/100T
C1067S/M	Annexin V-EGFP Apoptosis Detection Kit	20/50T
C1071S/M	Mitochondrial Membrane Potential and Apoptosis Detection Kit	20/50T
C1086/C1088	One Step TUNEL Apoptosis Assay Kit (Green Fluorescence)	20/50T
C1089/C1090	One Step TUNEL Apoptosis Assay Kit (Red Fluorescence)	20/50T
C1091/C1098	Colorimetric TUNEL Apoptosis Assay Kit	20/50T
C2006	Mitochondrial membrane potential assay kit with JC-1	>100T
C2009S	Mitochondrial Permeability Transition Pore Assay Kit or MPTP Assay Kit	100-1000T
C0009	MTT Cell Proliferation and Cytotoxicity Assay Kit	500T
C0013	Neutral Red Cell Proliferation and Cytotoxicity Assay Kit	500T
C0016/C0017	LDH Cytotoxicity Assay Kit	100/500T
C0035	WST-1 Cell Proliferation and Cytotoxicity Assay Kit	100T
C0036	WST-1 Cell Proliferation and Cytotoxicity Assay Kit	500/2500T
C0037-C0040	Cell Counting Kit-8	100-10000T
C0041-C0046	Enhanced Cell Counting Kit-8	100-10000T
C0065S/M/L/XL	CellTiter-Lumi™ Luminescent Cell Viability Assay Kit	100-10000T
C0068S/M/L/XL	CellTiter-Lumi™ Plus Luminescent Cell Viability Assay Kit	100-10000T
C2015S/M/L	Calcein/PI Cell Viability/Cytotoxicity Assay Kit	100/500/2500T

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