Cell Counting Kit-8 with Annexin V, FITC Assay Kit

Cytotoxicity Assay and Apoptosis Assay

General Information

The kit contains Cell Counting Kit-8 for cell proliferation assay and Annexin V, FITC Kit for apoptosis detection. The advantage of WST-8* dye is a characteristic of low toxicity to the cells, and it allows to combine cell viability assay based on WST-8 (CellCounting Kit-8) and apoptosis assay (Annexin V, FITC Kit). This combinational assay leads two sets of data from the same cell sample after treating the cells. The result gives not only viability change, but also it gives the cause of cell viability change that can be from the dehydrogenase activity loss or presence of apoptotic cells. [*Patent No. US 6,063,587 EP 0908453 JP 2757348 Canada 2,251,850]

CCK-8 is an one-bottle solution; no premixing of components is required. CCK-8, being nonradioactive allows sensitive colorimetric assays to determine the number of viable cells in cell proliferation and cytotoxicity assays. WST-8* is reduced by dehydrogenases in the cells giving an orange colored formazan, which is soluble in the tissue culture medium (Figure 1). The amount of formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. Especially, since the toxicity of CCK-8 is so low, the same cells can be used for other cell based assays such as apoptosis assay and DNA fluorometric assay after the CCK-8 assay is completed. [*Patent No. US 6,063,587 EP 0908453 JP 2757348 Canada 2,251,850]

Annexin V stained cells are used to indicate cell membrane changes that occur in the early stage of apoptosis. Once apoptosis is initiated, the phosphatidylserine present in the inner cell membrane migrates through the cell membrane of the lipid bilayer. Annexin V specifically binds to phosphatidylserine in the presence of protein-dependent Ca ion. By using fluorescent labeled Annexin V, the apoptotic cells can be identified using flow cytometry or fluorescence microscopy. Due to the different wavelength characteristics of FITC labeled Annexin V and PI nuclei stain, it is possible to perform double staining simultaneously and observe the stages of apoptosis in the cell membrane(Figure 2).

Kit Contents

[AC10-01]
- Cell Counting Kit-8 : 100 tests *1
  1) Cell Counting Kit-8: 1 mL bottle x 1
- Annexin V, FITC Apoptosis Detection Assays Kit : 20 Assays *2
  1) Annexin V, FITC Conjugate: 20 Assays x 1
  2) PI Solution: 50 Assays x 1
  3) Annexin V Binding Buffer: 50 Assays x 1

[AC10-05]
- Cell Counting Kit-8 : 500 tests *1
  1) Cell Counting Kit-8: 5 mL bottle x 1
- Annexin V, FITC Apoptosis Detection Assays Kit : 100 Assays *2
  1) Annexin V, FITC Conjugate: 100 Assays x 1
  2) PI Solution: 50 Assays x 2
  3) Annexin V Binding Buffer: 50 Assays x 2

*1 One test corresponds to one well on a 96-well plate.
*2 One assay corresponds to assay with cell concentration of 1 x 10^6 cells / ml.

Storage

CCK-8 is stable for over one year at 0-5°C with protection from light. Annexin V, FITC Kit is stable for 6 months at 0-5°C with protection from light.

Required Equipment and Materials

Cell Counting Kit-8
- plate reader (450 nm filter)
- 96-well plate
- CO2 incubator
- 10 μl and 100 - 200 μl multi-channel pipettes

Annexin V, FITC Apoptosis Detection Assays Kit
- Adjustable pipettes
- Flow cytometer or fluorescence microscope
  *Approximate fluorescence maximum excitation/emission:
  Annexin V, FITC: 494 nm / 518 nm; PI: 535 nm / 617 nm
- Phosphate buffered saline (PBS)
- Deionized water
General Protocol

Cell Counting Kit-8 Assay and Annexin V, FITC Assay are independent assay from each other. When performing Cell Counting Kit-8 Assay and Annexin V, FITC Assay, please follow the following general protocol.

**Cell Counting Kit-8 Assay for Cell Proliferation**

*Please see the technical manual of "Cell Counting Kit-8".

After measuring the absorbance with the microplate reader, use the same plate(cells) to perform Annexin V, FITC Assay immediately. The medium with Cell Counting Kit-8 should have orange coloring.

**Annexin V, FITC Assay for Apoptosis Detection**

*Please start from step 1. of Annexin V Staining General Protocol on the technical manual of "Annexin V, FITC Apoptosis Detection Kit".

Data

Continuous culture is possible without killing cells.

**Figure 3.** Observation of HeLa cells at 24 hours after the addition of WST-8(CCK-8).

**Figure 4.** The number of viable cells after induction of apoptosis.
- Cells: Jurkat cell (1x10⁵ cells/well)
- Induction of Apoptosis: Staurosporine 3.5 hours (5%CO₂, 37 °C)
- After the induction of apoptosis, cell proliferation was measured using Cell Counting Kit-8.

**Figure 5.** The number of viable cells after induction of apoptosis.
- Cells: MDA-MB-231 cell (2x10⁵ cells/well)
- Induction of Apoptosis: Paclitaxel (μmol/l) 4 days (5%CO₂, 37 °C)
- After the induction of apoptosis, cell proliferation was measured using Cell Counting Kit-8.

Cells after the cell proliferation assay were used for induction of apoptosis (dark line) and cells without performing the cell proliferation assay were used for induction of apoptosis (light line). As a result, similar readings were observed, which confirms that the same cells can be reused for induction of apoptosis after the cell proliferation assay.

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