03285 Fluorescent cell counting kit

Application

Fluorescent Cell Counting Kit (FCCK) is utilized for the fluorometric detection of living cell numbers. The amount of a fluorescent dye, calcein, produced from Calcein-AM (3',6'-Di(O-acetyl)-2',7'-bis[N,N-bis-(carboxymethyl) aminomethyl]-fluorescein, tetraacetoxymethyl ester) by esterases in cells is directly proportional to the number of viable cells in a culture medium. Since Calcein-AM is highly lypohilic because of the acetoxymethy groups in the molecule, it can rapidly permeate into the cytoplasm through the cell membrane. The FCCK assay does not require any radioisotopes (such as in the [3H]- thymidine incorporated assay) or a solubilization procedure (such as in the MTT assay). Therefore, it allows the users to obtain highly reproducible and accurate cell proliferation assay results.

Equipment required

1. Fluorescence microplate reader (excitation filter: 490 ± 10 nm, emission filter: 530 ± 15 nm)
2. 10 µl, 100 µl pipettes, multi-channel pipette
3. 96-well black plate or white plate
4. Equipments for cell culturing
5. Microplate centrifuge (for non-adhesive cells only)
6. Dulbecco's Phosphate Buffered Saline without Ca and Mg (D-PBS (-))

Preparation of working solution

Mix 100 µl of FCCK reagent with 5 ml of D-PBS (-) prior to use (1/50 dilution). Use 10 µl of the FCCK/D-PBS (-) solution for 100 µl cell culture. 5 ml FCCK working solution is sufficient for 5 plates (96-well). The FCCK working solution should be used up in one day.

Methods

Cell proliferation assay for adhesive cells

1. Wash each well of the 96-well plate with D-PBS (-) several times to remove esterase and phenol red. Leave 100 µ of D-PBS (-) in each well.
2. Add 10 µl of FCCK working solution to each well.
3. Incubate the plate for 30 min in the incubator.
4. Measure the fluorescence intensity of each well at 535 nm (excitation at 485 nm) using a fluorescence plate reader.

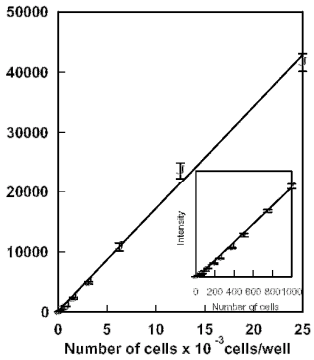
Cell proliferation assay for non-adhesive cells

1. Spin down cells on 96-well plate with a microplate centrifuge (1000 rpm, 5 min).
2. Discard the supernatant and add D-PBS (-) to wash cells.
3. Repeat steps 1 and 2 several times to remove esterase and phenol red.
4. Add 100 µl D-PBS (-) to each well and pre-incubate in an incubator.
5. Add 10 µl of FCCK working solution to each well of the plate.
6. Incubate the plate for 30 min in the incubator (longer incubation time may be required).
7. Measure the fluorescence intensity of each well at 535 nm (excitation at 485 nm) using a fluorescence plate reader.

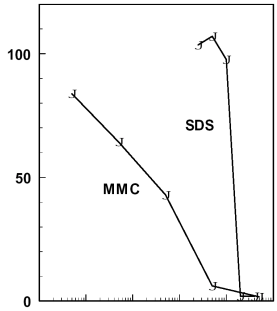
Cytotoxicity Assay

1. Dispense 100 µl of cell suspension (5000 cells/well) onto a 96-well microplate.
2. Pre-incubate the plate for 24 hours in an incubator.
3. Add 10 µl of various concentrations of a toxicant into the culture medium of the plate.
4. Incubate the cell cultures for 48 hours in the incubator.
5. Discard the medium, and wash the cells with 100 µl PBS (-): use a microplate centrifuge in the case of non-adhesive cells. Leave 100 µl of D- PBS (-) in each well.
6. Add 10 µl of FCCK working solution, and incubate the cell cultures for 30-60 min in the incubator.
7. Measure the fluorescence intensity of each well at 535 nm (excitation at 485 nm) using a fluorescence plate reader.

Cell proliferation assay (HLA cells)
RPMI1640 medium, 10% FCS, L-glutamine
Staining: 37 °C, 5% CO2, 30 min.
Detection: ☐ex = 485 nm, ☐em = 535 nm



Toxicological test (HL60-cells)
RPMI1640, 10% FCS, L-glutamine
Chemicals: Mitomycin C (MMC)
Dodecylsulfate, sodium salt (SDS)
Staining: 37 °C, 5% CO2, 30 min
Detection: λex = 485 nm, λem = 535 nm



Storage

Reagent solution is stable for 12 months at -20 °C with protection from light and moisture. Since the buffer solution of Calcein-AM is gradually hydrolyzed to generate fluorescent Calcein, the FCCK working solution is not storable. Close the bottle cap tightly after using a portion of FCCK to avoid moisture.

Notes

1. Since Calcein-AM is hydrolyzed with water, please close the bottle cap tightly after using the solution to avoidmoisture.
2. Since the buffer solution of Calcein-AM is gradually hydrolyzed to generate fluorescent Calcein, the FCCK working solution is not storable. Please use up the solution in one day.
3. Since the phenol red and serum in a culture medium interfere with the FCCK fluorescence measurement, the washing process with D-PBS (-) is important.
4. The incubation time may vary with individual experiment settings (e.g., cell type and number of cells in a well). Generally, non-adhesive cells (leucocytes) give a weak fluorescence, so a longer incubation time may be needed.
5. White plate (for luminescence detection) is recommended if you work with non-adhesive cells.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.