Revised May 26, 2004

Fluo Cell Counting Kit

Technical Manual - #MFP-C199

Contents

GENERAL INFORMATION

Fluo 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 (Figure 1). Since Calcein-AM is highly lypohilic because of the acetoxymethyl 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 [^{3}H]-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.

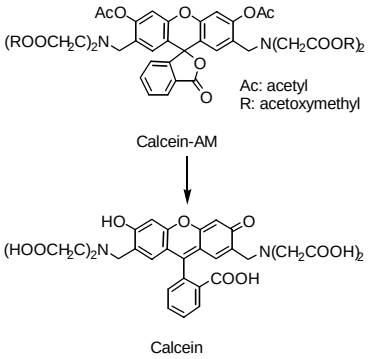

Figure 1. Structure of Calcein-AM and Calcein

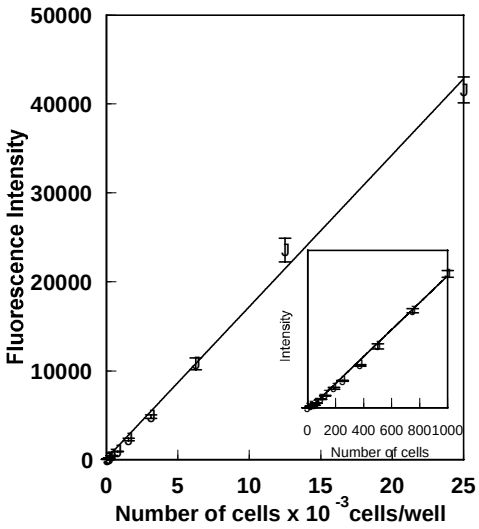

Figure 2. Cell proliferation assay using FCCK

Cell line: HL60
Culture medium: RPMI1640, 10% FCS, L-glutamine
Staining: 37 °C, 5% CO_2, 30 min.
Detection: λ_{ex} = 485 nm, λ_{em} = 535 nm

The FCCK detection range for the number of viable cells is from 50 or fewer to at least 25000 cells as shown in Figure 2. The cell proliferation assay using FCCK correlates well with the [^{3}H]-thymidine incorporated assay (Figure 3). Therefore, the FCCK assay can be substituted for the [^{3}H]-thymidine incorporated assay. Since serum and phenol red interfere with the fluorescent measurement, replacing a cell culture medium with D-PBS (-) prior to adding the FCCK solution is necessary.

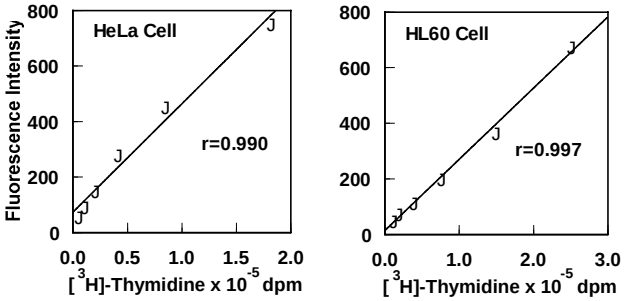

Figure 3. Correlation between the [^{3}H]-thymidine incorporated assay and the FCCK assay.

Cell line: HeLa, HL60
Culture medium: MEM, 10% FCS (HeLa)
RPMI1640, 10% FCS (HL60)
Reagent: [^{3}H]-thymidine: 37 KBq/well
FCCK: 10 µl/well
Reaction: [^{3}H]-thymidine assay: 4 h.
FCCK assay: 30 min.

ADVANTAGES

- No radioisotope is required.
- More sensitive than colorimetric assay using tetrazolium salt.
- Short staining reaction time and no solubilization step.

STORAGE

FCCK 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.

HOW TO USE THE KIT

1. Required Equipments and Materials

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

2. Preparation of the FCCK working solution

Mix 100 µl of FCCK 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.

3. Protocol

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 µl of D-PBS (-) in each well.
2) Add 10 µl of FCCK working solution to each well.
3) Incubate the plate for 30 minutes in the incubator.
4) Measure the fluorescence intensity of each well at 535 nm (excitation at 485 nm) using a fluorescence plate reader.

For non-adhesive cells:

1) Spin down cells on 96-well plate with a microplate centrifuge (1000 rpm, 5 minutes).
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 minutes 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.

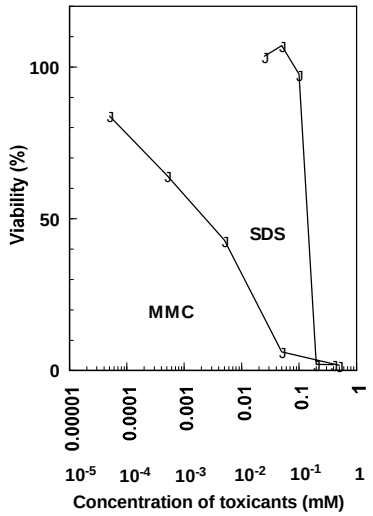

Figure 4. Toxicological test of chemicals using FCCK

Cell line: HL60
Culture medium: RPMI1640, 10% FCS, L-glutamine
Chemicals: Mitomycin C (MMC)
Dodecylsulfate, sodium salt (SDS)
Staining: 37 °C, 5% CO_2, 30 min
Detection: λ_{ex} = 485 nm, λ_{em} = 535 nm

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.

NOTES

1. Since Calcein-AM is hydrolyzed with water, please close the bottle cap tightly after using the solution to avoid moisture.
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.

PRODUCT CODE AND PRICE

Product	Unit	Product code	Price
FCCK*	500 tests	MFP-C199	

* Fluo Cell Counting Kit, Content: Calcein-AM solution 110 µl, 1 vial

RELATED PRODUCTS

Product	Unit	Product code	Price
Calcein-AM	1 mg	MFP-C1430	
Calcein-AM	1 ml (1 mM)	MFP-C3099	

For technical questions, please contact us at info@mobitec.com.

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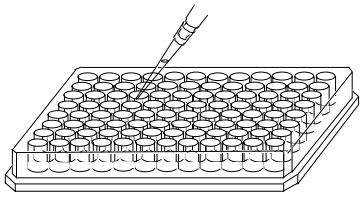
General Protocol at a Glance (for Adhesive Cells)

Read Technical Information carefully prior to using this General Protocol

Step 1)

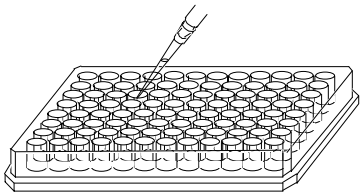
Prepare FCCK working solution[a)].
Remove the culture medium from each well, and wash cells with 200 µl D-PBS (-)[b)] several times.[c)]



a) dilute 100 µl FCCK with 5 ml D-PBS (-).
5 ml is a sufficient amount for 5 plates
the FCCK working solution should be used up in one day
b) D-PBS (-): Dulbecco's Phosphate Buffered Saline without Ca and Mg.
c) washing process is required to remove esterase and Phenol red.

Step 2)

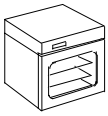
Add 100 µl D-PBS (-) to each well, and add 10 µl of FCCK working solution.

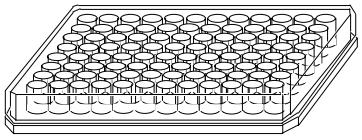


Step 3)

Incubate the plate in a CO_2 incubator for 30 min.

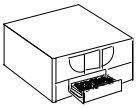
30 min.





Step 4)

Put the plate in a fluorescence microplate reader, and read the fluorescence intensity at 535 nm (excitation at 485 nm). Determine the viable cell number in a sample medium using the calibration curve prepared by the use of solutions containing known numbers of viable cells, or determine LD_{50} of toxicant used.

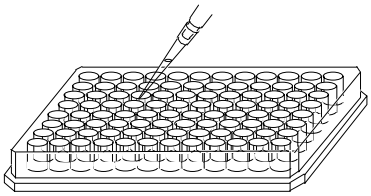


General Protocol at a Glance (for Non-adhesive Cells)

Read Technical Information carefully prior to using this General Protocol

Step 1)

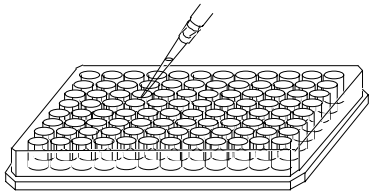
Prepare FCCK working solution[a)].
Spin the plate and remove supernatant of the culture medium from each well and add 200 µl D-PBS (-).[b)] Repeat this step several times.[c)]



a) dilute 100 µl FCCK with 5 ml D-PBS (-).
5 ml is enough for 5 plates
FCCK working solution should be used up in one day
b) D-PBS (-): Dulbecco's Phosphate Buffered Saline without Ca and Mg.
c) washing process is required to remove esterase and Phenol red.

Step 2)

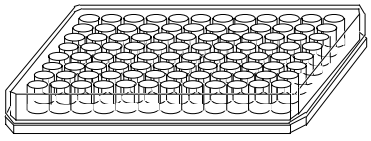
Add 100 µl D-PBS (-) to each well, and add 10 µl of FCCK working solution.



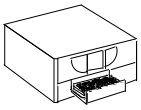
Step 3)

Incubate the plate in a CO_2 incubator for 30 min.

30 min.



Step 4)



Put the plate in a fluorescence microplate reader, and read the fluorescence intensity at 535 nm (excitation at 485 nm). Determine the viable cell number in a sample medium using the calibration curve prepared by the use of solutions containing known numbers of viable cells, or determine LD_{50} of toxicant used.