Cell Counting Kit-F (500 tests) Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/ck06.pdf

Introduction

Cell Counting Kit-F(CCK-F) offers simplified and highly sensitive cell proliferation and cytotoxicity assay method by employing Calcein-AM(3',6'-Di(*O*-acetyl)-4',5'-bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein, tetraacetoxymethyl ester) that produces a highly sensitive fluorescent dye(λ_{ex}=490 nm, λ_{em}=515 nm)upon enzymatic hydrolysis by esterases in living cells. The amount of the fluorescent dye, calcein, produced by hydrolysis by esterases in cells is directly proportional to the number of viable cells in a culture medium (Fig. 1).
Furthermore, CCK-F assay does not require any radioisotopes(such as in [^{3}H]-thymidine incorporation assay)nor solubilization procedure(such as in MTT assay),and no special skills are necessary for use. Therefore, it allows you to obtain highly reprodutive and accurate results.

Kit Contents

- Calcein-AM DMSO solution 110 µl × 1

Required equipment and materials

- Fluorescence microplate reader (excitation filter: 480-500 nm, emission filter: 500-535 nm)
- 96-well microplate for fluorescent measurements (black plate or white plate)

Storage

Store at -20℃ . This product is stable for six months at -20℃ before opening.
If the solution remains, close the bottle cap tightly and store in a freezer at -20℃ .

Working solution

Required amount of Calcein-AM DMSO solution is diluted 50 times with PBS(-). Since Calcein-AM is not stable in PBS(-), prepare the working solution immediately before use.

Cytotoxicity assay procedure

1. Dispense 100 µl of cell suspension (5,000 cells/well) onto a 96-well microtiter plate.
2. Preincubate the plate for 24 hours in an incubator (humidified atmosphere, e.g.,at 37℃ , 5% CO_2).
3. Add 10 µl of various concentrations of the test substance into the culture medium of the plate.
4. Incubate cell cultures for 48 hours in the incubator.
5. Replace the medium with 100 µl of PBS(-). Use a centrituge for microplate in the case of nonadherent type cells before removing the culture medium.
6. Add 10 µl of the working solution and incubate cell cultures for 15-30 min in the incubator.
7. Measure the fluorescent(λ_{ex}=490 nm, λ_{em}=515 nm) using a fluorescence microplate reader.

Determination of IC_{50}

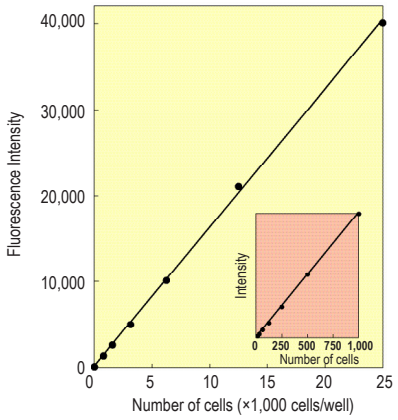
Cell viability is calculated using the following equation. IC_{50}, concentration killing 50% of the cells, is determined from plot of viability versus concentration of the test substance.

Viability (%) = $[(A_s-A_b)/(A_c-A_b)] \times 100$
A_s: Fluorescence intensity of sample (cell + test substance + CCK-F)
A_c: Fluorescence intensity of control (cell + CCK-F, no test substance)
A_b: Fluorescence intensity of blank (medium + CCK-F, no cell)

Example of toxicological test using CCK-F is shown in Fig. 3.

Caution

1. Phenol red and serum in a culture medium interfere with the fluorescence measurement. Replace a culture medium with PBS(-) or phenol red and serum free medium prior to adding the working solution.
2. Dilute the test substance with non-toxic solution, such as culture medium or PBS(-) or saline. And use same solution for control and blank wells instead of sample.
3. If a 24-well or 6-well plate is used for this assay, calculate the number of cells per well accordingly, and adjust the volume of the working solution in a well to 10% of the total volume.



Cell line: HL60
Incubation time: 30 min
Detection: λ_{ex}=485 nm, λ_{em}=535 nm

Fig.1 A relationship between fluorescence intensity and number

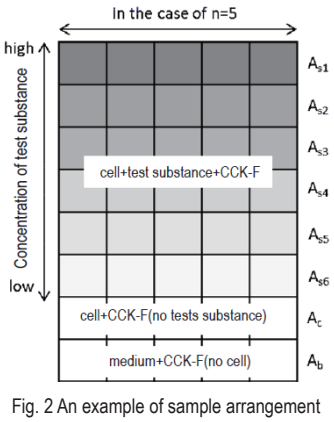
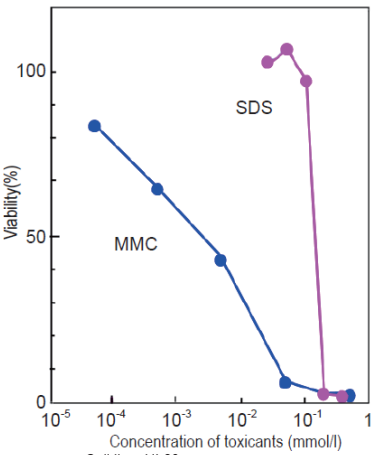


Fig. 2 An example of sample arrangement



Cell line: HL60
Toxicants: Mitomycin C (MMC),
Sodium dodecylsulfate(SDS)
Condition: 37℃ , 5% CO_2, 48 hrs
Incubation time: 30 min
Detection: λ_{ex}=485 nm, λ_{em}=535 nm

Fig.3 Toxicological test for MMC and SDS by CCK-F.

If you need more information, please contact Dojindo technical service.

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