Product Manual

Cell Counting Kit-F (500 tests)

ALX-850-245-KI01: ~500 tests

ALX-850-245-KI02: 5x ~500 tests

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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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 (λex=490 nm, λem=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.

The CCK-F assay has a detection range from 50 or fewer to at least 25,000 cells. Furthermore, CCK-F assay does not require any radioisotopes (such as in [3H]-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 reproductive and accurate results.

MATERIALS SUPPLIED

Calcein-AM DMSO solution, 110 µl×1

Reagents require separate storage conditions.

STORAGE

Store at -20°C. This product is stable for six months at -20°C before opening.

If the solution remains, close the bottle cap tightly and store in a freezer at -20℃.

OTHER MATERIALS NEEDED

1. Fluorescence microplate reader (excitation filter: 480-500 nm, emission filter: 500-535 nm)
2. 96-well microplate for fluorescent measurements (black plate or white plate)
3. Phosphate Buffered Saline (PBS)

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°C, 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. For nonadherent cells, centrifuge the microplate 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 fluorescence (λex=490 nm, λem=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)

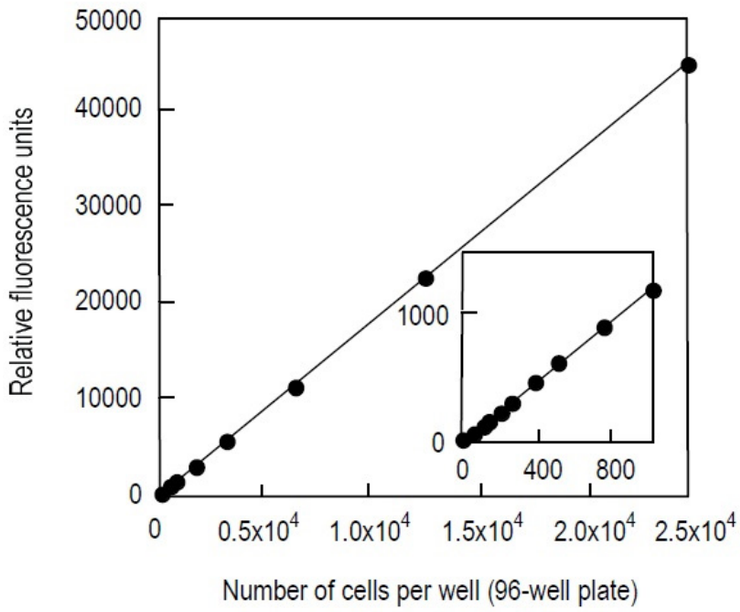
A_b: Fluorescence intensity of blank (medium + CCK-F, no cell)

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.

TYPICAL RESULTS



Cell line: HL60
Incubation time: 30 min
Detection: λex=485 nm, λem=535 nm

Fig.1 A relationship between fluorescence intensity and number

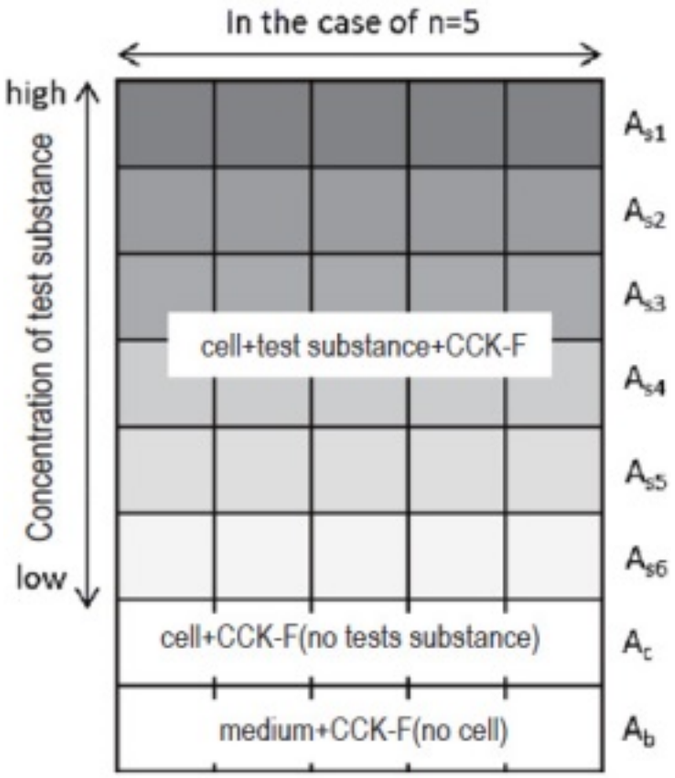
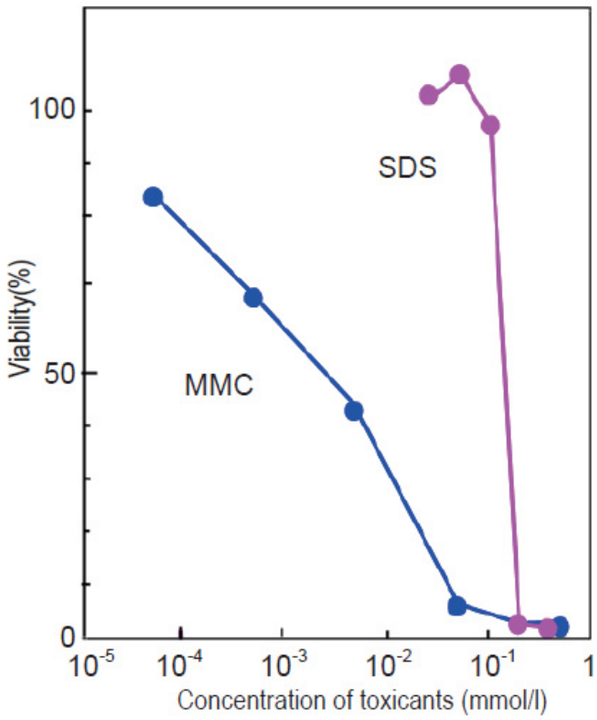


Fig. 2 An example of sample arrangement



Cell line: HL60
Toxicants: Mitomycin C (MMC), Sodium dodecylsulfate (SDS)
Condition: 37℃, 5% CO2, 48 hrs
Incubation time: 30 min
Detection: λex=485 nm, λem=535 nm

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

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