TransDetect® Cell Counting Kit (CCK)

Cat. No. FC101

Storage: at 2-8°C in dark for one year or at -20°C in dark for two years

Description

TransDetect® Cell Counting Kit (CCK) is designed for cell proliferation assays as well as cytotoxicity assays by utilizing a water-soluble tetrazolium salt. The salt can be reduced to an orange water-soluble formazan by mitochondrial dehydrogenase in the presence of an electron coupling reagent 1-Methoxy PMS. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. Faster cell proliferation, lower cytotoxicity, and more cell number produce deeper color. The depth of the dye is directly proportional to the number of living cells. The toxicity of CCK solution is so low that the same cells can be used for other assays after the CCK assay is completed. Compared with MTT, XTT, MST and WST-1, this method provides higher sensitivity and broader linear range. It is suitable for drug screening, cell proliferation test, cytotoxicity assay and drug sensitivity test.

Kit Contents

Component	FC101-01	FC101-02	FC101-03	FC101-04
CCK Solution	1 ml	5 ml	10 ml	30 ml

Procedure

1. Inoculate cell suspension in a 96-well plate (100 µl/well). Pre-incubate the plate in a cell incubator at 37°C for 12-24 hours according to experimental need. In general, use 2×10^3 cells per well for cell proliferation assays, use 5×10^3 cells per well for cytotoxicity assays.
2. Add appropriate volume (0-10 µl) of substance to be tested to the plate, incubate for an appropriate length of time in the incubator.
3. Add CCK solution (equal to 1/10 of the media volume) to each well of the 96-well plate (e.g. add 10 µl of CCK solution for 100 of the media).
4. Incubate the plate for 1-4 hour in the incubator.
5. Measure the absorbance at 450 nm using a microplate reader.

Notes

1. The presence of phenol red has no effect on the result, but can increase the background absorption. Thus the blank absorbance should be subtracted.
2. The incubation time after adding CCK solution varies by the cell type and density. Perform initial experiments to determine the appropriate incubation time. Generally, lymphocyte has lower sensitivity, which requires longer incubation time or higher cell density.
3. Assays by this kit depend on the catalyzation of dehydrogenase in cells. If the substance to be tested has strong oxidativity or reductivity, fresh media should be changed prior to adding CCK solution.
4. Be careful not to introduce bubbles to the wells since they will interfere with absorbance value.
5. If there is no 450 nm optical filter, the filter with absorbance between 430 nm and 490 nm can be used.
6. For highly turbid cell suspension, 600 nm (or above 600 nm) can be used as a reference to perform dual wavelength measurement.
7. To stop the reaction, add 10 µl of 0.1 M HCl or 1% w/v SDS solution into each well of 96-well plate, and store in dark. The absorbance will not change within 24 hours.
8. The toxicity of CCK solution is so low that the same cells can be used for other assays after the CCK assay is completed.
9. This kit should be stored in dark. For long-term storage, aliquot CCK solution and store at -20°C.

FOR RESEARCH USE ONLY