Cell Counting Kit-8 (CCK-8) Assay

(Cat. No.: S1GNM03j30003)

Description:

The Cell Counting Kit-8 (CCK-8) assays through the use of the highly water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt], which produces a water-soluble formazan dye when reduced in the presence of an electron mediator, as illustrated in Fig. 1. CCK-8 is provided as a one-bottle solution, eliminating the need for pre-mixing components. This non-radioactive Cell Counting Kit-8 facilitates sensitive colorimetric assays for determining the count of viable cells in cell proliferation and cytotoxicity experiments.

WST-8 undergoes reduction by cellular dehydrogenases, yielding an orange-colored formazan product that remains soluble in the culture medium. The quantity of formazan dye generated by cellular dehydrogenase activity is directly proportional to the number of viable cells. The detection sensitivity of CCK-8 surpasses that of other tetrazolium salts such as MTT, XTT, MTS, or WST-1.

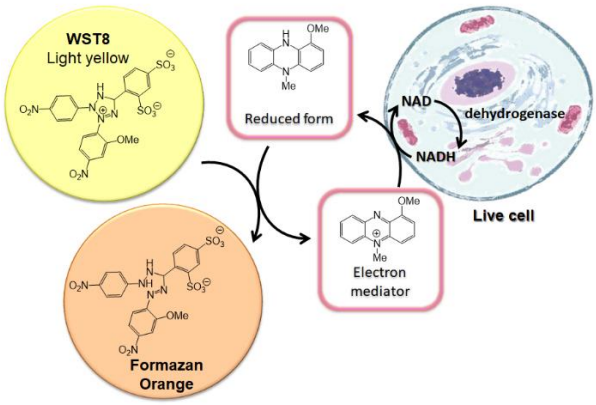

Fig 1. Principle of the cell viability detection with CCK-8 assay.

Kit Contents:

Contents	S1GNM03j30003 (500 rxns)
Cell Counting Kit-8 (CCK-8)	500 rxns x 1

Storage:

For long-term storage, the CCK-8 solution remains stable for 12 months at -20°C when protected from light. If used frequently, it can be stored at 0-5°C, shielded from light, and should be used within one week after opening.

Precautions:

This product should maintain its original red color; do not use it if the solution has turned yellow. Repeated thawing and freezing can result in an increase in background noise, which interferes with the assay. Always ensure that the CCK-8 is protected from exposure to light.

Protocol:

✧ ***If the reagent assay is frozen, before use, it must be taken out of the -20°C refrigerator, placed in a refrigerated environment at 4-8°C and stored for 4 hours or at room temperature for 30 minutes.***

1. Dispense 100 µl of cell suspension per well into a 96-well plate. Pre-incubate the plate overnight in a humidified incubator (e.g., at 37 °C, 5% CO_2).
2. Gently add 10 µl of the thawed CCK-8 solution to each well in the plate. Exercise caution to prevent the introduction of bubbles into the wells, as they can interfere with optical density (O.D.) readings.
3. Protect from the light and Incubate the plate for 1 to 4 hours at 37°C with shaking.
4. Utilize a microplate reader to measure the absorbance at 450 nm.

Background control

Spontaneous absorbance around 450 nm may arise in the culture medium that is incubated with CCK-8. This inherent background absorbance is influenced by factors such as the culture medium itself, pH, incubation duration, and light exposure time. Typically, the background absorbance ranges from 0.1 to 0.2 absorbance units after a 2-hour incubation period. To address this, establish one or more control wells without cells and subtract the mean absorbance value of these control wells from the absorbance values of the remaining wells.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	S1GNM03j30003_Protocol_V1	Sep 2023