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GENLISA™ Cell Counting Kit-8 (CCK-8) Assay

REF : KBCA1266

Ver 1.0

RUO

Biochemical Assay for the Quantitative Determination of Cell Counting Kit-8 (CCK-8) in serum, plasma and tissue samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KBCA1266**  **100 tests**

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Introduction:

The CCK - 8 cell viability assay kit contains WST - 8 [2-(2 - methoxy - 4 - nitrophenyl)-3-(4 - nitrophenyl)-5-(2,4 - disulfonylbenzene) - 2H - tetrazolyl monosodium salt]. In the presence of electron carriers, intracellular dehydrogenases oxidize and reduce WST - 8, generating a water-soluble orange-yellow formazan dye that can dissolve in the tissue culture medium. The quantity of formazan produced is directly proportional to the number of viable cells. The sterile CCK - 8 solution can be added directly to the cell culture medium without the necessity of pre - mixing with other components. The CCK - 8 method represents a highly sensitive, non - radioactive colorimetric assay for quantifying the number of viable cells in cell proliferation or toxicity experiments, and it serves as a viable alternative to the traditional MTT method.

Intended Use:

GENLISA™ Cell Counting Kit-8 (CCK-8) Assay is to assess cell viability, proliferation, and cytotoxicity in various biological experiments.

Principle:

The CCK - 8 cell viability assay kit contains WST - 8 [2-(2 - methoxy - 4 - nitrophenyl)-3-(4 - nitrophenyl)-5-(2,4 - disulfonylbenzene) - 2H - tetrazolyl monosodium salt]. In the presence of electron carriers, intracellular dehydrogenases oxidize and reduce WST - 8, generating a water-soluble orange-yellow formazan dye that can dissolve in the tissue culture medium. The quantity of formazan produced is directly proportional to the number of viable cells. The sterile CCK - 8 solution can be added directly to the cell culture medium without the necessity of pre - mixing with other components. The CCK - 8 method represents a highly sensitive, non - radioactive colorimetric assay for quantifying the number of viable cells in cell proliferation or toxicity experiments, and it serves as a viable alternative to the traditional MTT method.

Materials Provided:

1. Reagent I – CCK – 8 Solution - 1ml (100 tests)
2. Microwell Plate (100 tests) - 1 no.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Materials & Equipment Required But Not Provided:

1. Microplate Reader or Visible Spectrophotometer capable of measuring absorbance at 450 nm.
2. CO_2 Incubator, Ice Maker, Low-speed Centrifuge.
3. Plate shaker
4. 96 well Plate or Microglass cuvette, Precision pipettes, Disposable pipette tips.
5. Deionized water
6. Dounce homogenizer.

Assay Procedure:

1. Dispense 100 ul of the cell suspension (containing approximately 1×10^4 cells) into each well of a 96 - well plate. Conduct pre - culture of the plate in an incubator maintained at 37°C with 5% CO_2 for 24 hours.

2. Add 100 ul of either complete culture medium or complete culture medium supplemented with test samples at various concentrations to each well of the culture plate.

3. Continue culturing the culture plate in the incubator for an appropriate duration (e.g., 48 hours or 72 hours).

4. Pipette 10 ul of CCK - 8 solution into each well, taking care to avoid the formation of bubbles within the wells.

5. Incubate the culture plate in the incubator for a period ranging from 1 to 4 hours.

6. Measure the absorbance at 450 nm using a microplate reader.

7. In the event that immediate measurement of the OD value is not required and a subsequent measurement is planned, add 10ul of 0.1 M HCl solution or 1% (W/V) SDS solution to each well. Cover the culture plate and store it at room temperature in the dark. The absorbance will remain stable within a 24 - hour period.

Data Analysis and Interpretation of Results:

1. Cell Survival Rate Calculation: Subtract the background OD value (measured in wells containing complete medium and CCK - 8 but no cells) from the OD value of each test well. Subsequently, calculate the mean ± standard deviation (SD) of the OD values obtained from replicate wells.

   The cell survival rate is expressed as T/C%, where T represents the OD value of the drug – treated cells and C represents the OD value of the control cells.

   Cell survival rate (%) = (OD of drug - treated cells / OD of control cells) × 100

2. Calculation of Inhibitory Concentrations: Determine the drug concentration corresponding to T/C = 50% (IC50) or the drug concentration corresponding to T/C = 10% (IC90).

Precautions and Notes:

1. During cell inoculation, ensure thorough mixing of the cell suspension to prevent cell sedimentation, which may result in unequal cell numbers among wells. It is advisable to remix the suspension after inoculating several wells. The culture medium in the peripheral wells of the culture plate is prone to evaporation. To minimize experimental errors, it is recommended to fill the wells on the four sides of the culture plate with only culture medium or sterile PBS and exclude them from indicator detection.

2. The optimal reaction time of CCK - 8 varies depending on specific color - development requirements. For initial experiments, it is recommended to prepare multiple wells to determine the optimal cell seeding density and the optimal incubation time following the addition of the CCK - 8 reagent. Generally, white blood cells are more difficult to stain, necessitating either an extended CCK - 8 reaction time or an increased number of cells (approximately 10^5 cells/well). Suspension cells pose greater challenges in staining compared to adherent cells. For suspension cells, after adding CCK - 8 and incubating for 1 to 4 hours, remove the plate from the incubator to visually assess the staining degree or use a microplate reader to determine the optimal incubation time. If staining is insufficient, return the culture plate to the incubator and continue culturing for additional hours before measurement. For adherent cells, the typical incubation time for CCK - 8 ranges from 1 to 4 hours. Most adherent cells can be visually inspected after approximately 30 minutes of incubation, and optimal detection results are usually achieved after around 3.5 hours of incubation.

3. The detection mechanism of this kit relies on the dehydrogenase - catalyzed reaction. Excessive reducing agents present in the test system, such as certain antioxidants, may interfere with the detection process and should be eliminated. The presence of phenol red in the culture medium does not affect the determination of cell viability using this kit.

4. Assessment of Reducing Substances in the Test Solution: Add 10ul of CCK - 8 solution to a well containing only the test solution (without cells), incubate for 1 to 4 hours, and measure the blank absorbance at 450nm. A very low absorbance value indicates the presence of minimal reducing agents in the test system, allowing for the direct addition of CCK - 8 during the formal test. Conversely, a relatively high absorbance value suggests the presence of significant reducing agents. In such cases, for the formal test, remove the culture medium, wash the cells twice with fresh culture medium, and then add new culture medium and 10 ul of CCK - 8 for detection.

5. When dealing with cell suspensions of high turbidity, it is recommended to set 600 nm (or a wavelength above 600 nm) as the reference wavelength and subtract the OD value measured at this reference wavelength.

6. Appropriate personal protective equipment, including a lab coat and disposable gloves, should be worn during the experiment.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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