Protocol Booklet

Product Code(s)	HB9337
Product Name	Cell counting kit-8
Purpose	Colorimetric quantification of viable cell number

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Contents

Product Overview

Cell Counting Kit-8 (CCK-8) is a ready to use solution for cell viability assays and cell proliferation assays. The kit uses WST-8 tetrazolium salt which is reduced by dehydrogenases in living cells to give a brightly colored dye. The dye generated is directly proportional to the number of live cells enabling colorimetric quantitation of viable cell number.

Components & Storage

CCK-8 is a ready to use solution that can be added directly to cells in culture.

Store CCK-8 at 4°C protected from light. For longer term storage, CCK-8 can be stored at -20°C, but should be aliquoted to avoid freeze thawing.

To sterilize CCK-8 solution use a 0.2 µm membrane filter before use.

Protocol

The below protocols are for cells plated in 96 well plates. If using 24-well or 6-well plates adjust the volume of CCK-8 solution to 10% of the total volume in the well.

Cell number determination

1. Plate cells at 100 µL/well in a 96 well plate and pre-incubate in a humidified incubator (37°C, 5% CO_2).
2. Add 10 µL of CCK-8 solution to each well of the plate.
3. Incubate for 1-4 hours in the incubator. Incubation time varies on cell type and cell number.
4. Measure absorbance at 450nm in a microplate reader.

The absorbance can be measured up to 24 hours later by addition of 10 µL of 0.1 M HCl or 1% w/v SDS to each well. The plate should be kept covered and away from light at room temperature.

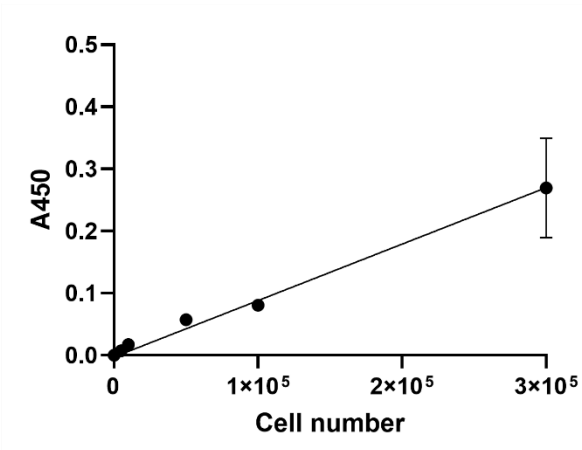

Figure 1: Example data of HEK293T cell number determined using Cell Counting Kit-8.

Cell proliferation and cytotoxicity assays

1. Plate cells at 100 µL/well at a density of 10^4 to 10^5 cells per well. Incubate cells in a humidified incubator (37°C, 5% CO_2) for 24 hours and add compounds to be tested at an appropriate timepoint.
2. Add 10 µL of CCK-8 solution to each well of the plate.
3. Incubate for 1-4 hours in the incubator. Incubation time varies on cell type and cell number.
4. Measure absorbance at 450nm in a microplate reader.

The absorbance can be measured up to 24 hours later by addition of 10 µL of 0.1 M HCl or 1% w/v SDS to each well. The plate should be kept covered and away from light at room temperature.

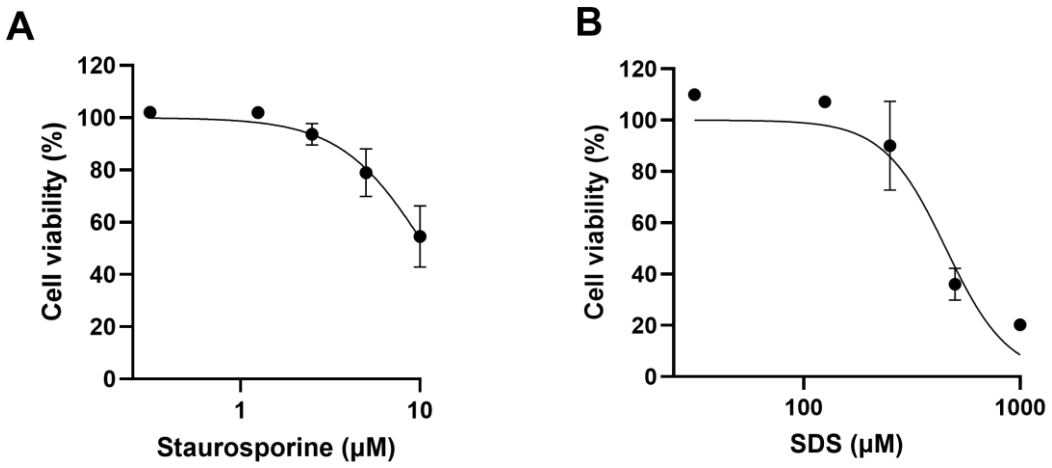

Figure 2: Example data of HEK293T cell viability upon treatment with different concentrations of (A) Staurosporine or (B) SDS measured using Cell Counting Kit-8.

Guidelines, precautions, troubleshooting

Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the assay kit.

Problem	Potential Cause
Weak signal	Incubation time not long enough. Incubation time is cell type dependent – for example, leukocytes give weak signal so a longer incubation time is required.
	Too few cells - for adhesive cells at least 1000 cells are necessary in 100 µL and for leukocytes at least 2500 cells are necessary in 100 µL.
High background	The dye in CCK-8 assay kit may react with reducing agents and cause a colorimetric chance. If using reducing agents check the background OD.
	High turbidity of the cell suspension may increase background. The OD can be read at 600 nm and this value can be subtracted from 450 nm readings.
Unexpected results	The CCK-8 assay uses dehydrogenase activity as a measure of cell viability. Therefore any cells, chemicals or conditions that may affect dehydrogenase activity may affect the CCK-8 assay.
	Bubbles are present in wells may affect the absorbance readings.

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