Cell Counting and Cytotoxicity Kit-8(CCK-8)

***Storage*：** 0-5°C

CCK-8 kit is stable over one year at 0-5°C with protection from light. Store it at -20°C for longer storage. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. Please store the kit at 0-5°C for frequent use.

Introduction：

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing Dojindo's highly water-soluble tetrazolium salt. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-*2H*-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron mediator

CCK-8 is a one-bottle solution; no premixing of components is required. CCK-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. WST- 8 is reduced by dehydrogenases in cells to give an orange colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells.

The cell proliferation assay using CCK-8 correlates well with the [$_3$H]-thymidine incorporation assay. Thus, the CCK-8 assay can also be substituted for the [$_3$H]-thymidine incorporation assay. As shown in Figure 4, the detection sensitivity using CCK-8 is higher than assays using other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Protocol：

Cell Number Determination

1. *Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37 °C, 5% CO_2).*
2. *Add 10 µl of the CCK-8 solution to each well of the plate.* *Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.*
3. *Incubate the plate for 1 - 4 hours in the incubator.*
4. *Measure the absorbance at 450 nm using a microplate reader.*

To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.

Cell Proliferation and Cytotoxicity Assay

1. Dispense 100 µl of cell suspension (5000-10000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO2).
2. Add 10 µl of various concentrations of substances to be tested to the plate.
3. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
4. Add 10 µl of CCK-8 solution to each well of the plate. *Be careful not to introduce bubbles to the wells, since they interfere with the O.D450 reading.*
5. Incubate the plate for 1 - 4 hours in the incubator.
6. Measure the absorbance at 450 nm using a microplate reader.

To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.

FAQ

1. How many cells should there be in a well?

For adhesive cells, at least 1000 cells are necessary per well (100 µl medium). For leukocytes, at least 2500 cells are necessary per well (100 µl medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25000. If a 24-well or 6-well plate is used for this assay, please calculate the number of cells per well accordingly, and adjust the volume of the CCK-8 solution in a well to 10% of the total volume.

2. Does CCK-8 stain viable cells?

No. Since WST-8 and its formazan dye are highly water-soluble, CCK-8 cannot be utilized for cell staining purpose.

3. Does phenol red affect the assay?

No. The absorption value of phenol red in a culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, a medium containing phenol red is usable for the CCK-8 assay.

4. Is CCK-8 toxic to cells?

Since the toxicity of CCK-8 is so low, the same cells can be used for other cell proliferation assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay after the CCK-8 assay is completed.

5. I do not have a 450 nm filter. What other filters can I use?

You can use filters with the absorbance between 430 and 490 nm, even though 450 nm filter gives the best sensitivity.

Cell culture experimental data(Only for reference)

	Cell		Cell quantity	Culture Media	CCK-8 volume µl	Time （hour)
1	A549	Adherent	8000	DMEM	10	2-3
2	AGS	Adherent	10000	F12	10	2
3	ASTC-a-1	Adherent	50000	RPMI1640	10	3.5
4	B16	Adherent	4000	DMEM	10	3
5	Balb/c 3T3	Adherent	10000	RPMI1640	10	4
6	Bcap-37	Adherent	5000	RPMI1640	10	4
7	Bel-7402	Adherent	5000	DMEM	10	2
8	CTLL-2	Adherent	16000	RPMI1640	10	3

9	EaHY926	Adherent	20000	DMEM	10	2
10	ECV340	Adherent	20000	DMEM	10	2
11	HaCaT	Adherent	10000	DMEM	10	4
12	HAEC	Adherent	10000	RPMI1640	10	4
13	HEK-293	Adherent	5000	DMEM	10	2
14	HepG2	Adherent	2000	DMEM	10	2
15	Hela	Adherent	2000-25000	MEM	10	2
16	Ho-8910	Adherent	80000	DMEM	10	3
17	HL60	Suspension	30000-45000	RPMI1640	10	3
18	HLF	Adherent	10000	DMEM	10	1
19	HS 766T	Adherent	100000	RPMI1640	10	2
20	Huh7	Adherent	100000	DMEM	10	2
21	HUVEC	Adherent	5000	RPMI1640	10	4
22	Hepatocytes	Adherent	30000	DMEM	10	2-3
23	HSC	Suspension	10000	DMEM	10	3
24	K562	Suspension	40000	RPMI1640	10	6
25	L1210	Suspension	200000	RPMI1640	10	3
26	L929	Adherent	1000	DMEM	10	4
27	MCF-7	Adherent	10000	RPMI1640	10	2-3
28	MN9D	Adherent	5000	DMEM/F12	10	4
29	NIH3T3	Adherent	10000	DMEM	10	1-2
30	NRK	Adherent	5000	DMEM	10	4
31	P815	Suspension	10000	RPMI1640	10	3
32	PC12	Adherent	10000	RPMI1640	10	3-4
33	QBC939	Adherent	5000	RPMI1640	10	4
34	Raji	Suspension	100000	RPMI1640	10	5
35	RAW264.7	Adherent	20000	DMEM	10	2
36	SGC-996	Adherent	150000	DMEM	10	2
37	SH-SY5Y	Suspension	12000	DMEM	10	3
38	SK	Adherent	80000	DMEM	50	3
39	SMC	Adherent	20000	DMEM	10	4
40	SMMC-7721	Adherent	1000	DMEM	10	2
41	SPC-A1	Adherent	3000	RPMI1640	10	3
42	SW480	Adherent	1000	DMEM	10	2
43	T-24	Adherent	80000	DMEM	10	3
44	Tca8113	Adherent	10000000	RPMI1640	10	4
45	T lymph cell	Suspension	10000-1000000	RPMI1640	10	2
46	U937	Suspension	200000	RPMI1640	10	3
47	Vero E6	Adherent	30000	RPMI1640	10	2
48	Wish	Adherent	30000	MEM	10	2
49	7901	Adherent	15000-	RPMI1640	10	4