Cell Proliferation Assay Cocktail

Cat #: KTD103-EN **Size:** 1 kit

	Cell Proliferation Assay Cocktail		
REF	**Cat #:** KTD103-EN	LOT	**Lot #:** Refer to product label
	Applications: Suitable for Cell Proliferation and Cytotoxicity Assay		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Cell proliferation is a process of increasing the number of cells by means of cell division, which will produce many important changes, including the synthesis of DNA, the increase of cell metabolism, the expression of proliferation-specific proteins and so on.Taylor[1] first detected cell proliferation by infiltrating thymidine analogues into newly synthesized DNA in 1957. After continuous optimization and improvement of EdU method, no denaturation step, maintaining the integrity of cell morphology and DNA, it is recognized as the most direct and accurate method to detect cell proliferation.The process of cell proliferation is accompanied by the increase of cell metabolic activity. CCK-8 method is based on the change of dehydrogenase activity in cell mitochondria and indirectly reflects the number of living cells through the color of reduction product methylene. It is the most widely used method for cell proliferation detection. Based on the new generation of AbFluor™ fluorescent staining technology, Abbkine has developed a cocktail set that can accurately detect cell proliferation directly at the DNA level, and at the same time achieve high-throughput detection of cell proliferation at the level of cell metabolism. The kit contains a patented cytotoxic positive control, which perfectly solves the current problem of non-standardization of proliferation test results; As a standardized product for cell proliferation research, it can meet the needs of most users for cell proliferation detection, and can also be applied to the detection of cytotoxicity. It is the best choice for cell proliferation/toxicity detection.

Materials Supplied and Storage Conditions

Cocktail components	Kit components	Size	Storage conditions
Cell Proliferation EdU Image Kit (Green Fluorescence)-100 T	EdU (10 mM)	100 µL	-20℃
	BSA Wash Solution (5×)	12 mL	-20℃
	AbFluor 488 azide	20 µL	-20℃, protected from light
	10×Reaction buffer	1 mL	4℃
	Copper Reagent	0.4 mL	4℃
	Reducing Agent	100 mg	-20℃
	DAPI (500×)	24 µL	-20℃, protected from light

Cell Counting Kit-8 (CCK-8)-2000 T	CCK-8	20 mL	-20℃, protected from light
Cytotoxicity Postive Control-100 T	Postive Control	100 μL	-20℃, protected from light

Reagent Preparation

10×Reducing Agent: Add deionized water to each vial (final concentration 100 mg/mL) and mix until the compound is dissolved completely. After using, aliquots any remaining solution at -20℃. When stored as directed, this stock solution is stable for up to 6 months. If the solution starts to develop a brown color, it has degraded and should be discarded.

1×BSA Wash Solution : Phosphate buffer (PBS) was added to the BSA Wash Solution (5×), and the BSA Wash Solution (5×) was diluted into 1×BSA Wash Solution (final concentration was 3% BSA/PBS), and mixed until the compound was completely dissolved. After use, the solution can be stably stored at -20℃ for 6 months.

Assay Procedure

Ⅰ. Positive Cytotoxicity induction (Optional)

For cultured cells to be induced, the Postive Control is added into the culture medium according to the volume ratio of 1:1000-1:4000. For the most part, after stimulating 30 min-4 h, obvious changes in cell morphology can be seen under the light microscope, which can be used to detect Cytotoxicity (It is recommended to adjust the concentration of Positive Control and the induction time for different cells).

Ⅱ. Cell Proliferation and Cytotoxicity Assay by CCK8

1. Dispense 100 μL of cell suspension (5×10^3 cells/well) in a 96-well plate. Pre-incubate the plate for 24 h in a humidified incubator (e.g., at 37℃, 5% CO_2).
2. Add 1-10 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 h) 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 OD reading.
5. Incubate the plate for 1-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density.
6. Measure the absorbance at 450 nm using a microplate reader.

Notes:

1. Since the CCK-8 assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity in viable cells may cause discrepancy between the actual viable cell number and the cell number determined using the CCK-8 assay.
2. The incubation time varies by the type and number of cells in a well. Generally, leukocytes give weak coloration, thus a long incubation time (up to 4 h) or a large number of cells (approximately 10^5 cells/well) may be necessary.
3. If the color or pH of culture media is changed due to long-time culture, please change the culture media when adding CCK-8.
4. The same cells can be used for other cell assays because of the low toxicity of CCK-8.

Ⅲ. Cell Proliferation and Cytotoxicity Assay by EdU

Note: The protocol below refers to a 96-well culture plate; adjust volumes accordingly for other plate formats. The assay volume is 100 μL.

A. Labeling of cells with EdU

Note: This assay was developed with HeLa cells and may need modified according to the particular cell type. An EdU concentration of 10 μM is a good starting concentration.

1. Seed cells on coverslips and grow them until the desired density. Treat cells with the desired method.
2. Prepare a 2×EdU solution (20 μM EdU) in serum-free medium from the 10 mM EdU stock solution.
3. Prewarm the 2×EdU solution, and then add an equal volume of the 2×EdU solution to the volume of media containing cells to be treated to obtain a 1×EdU solution. For example, for a final concentration of 10 μM (1×EdU solution), replace half of the media

with fresh media containing 20 μM EdU (2×EdU solution).

Note: It is not recommended to replace all of the media, because this could affect the rate of cell proliferation.

4. Incubate the cells for 2 h under conditions optimal for the cell type (According to the cell amplification time, the incubation time of general tumor cells was 2 h).

5. Proceed immediately to Step B: Cell fixation and permeabilization.

B. Cell fixation and permeabilization

1. After incubation, remove the media and add 0.1 mL of 3.7% formaldehyde in PBS to each well containing the coverslips. Incubate for 15 min at room temperature.

2. Remove the fixative and wash the cells in each well with 0.1 mL of 1×BSA Wash Solution for 5 min. Repeat three times.

3. Remove the solution, and then add 0.1 mL of 0.5% Triton X-100 in PBS (permeabilization solution) to each well. Incubate for 20 min at room temperature.

4. Remove the permeabilization buffer, and then wash the cells in each well with 0.1 mL of 1×BSA Wash Solution for 5 min. Repeat three times. Remove the solution.

5. Proceed immediately to Step C: EdU detection.

C. EdU detection

Note: In this protocol, 100 μL of the reaction mix per well are used. Also, smaller volumes can be used, as long as the reaction components are applied in the same ratios.

1. Prepare Click-iT reaction mix according to Next Table. It is important to add the ingredients in the order listed in the table; otherwise, the reaction will not proceed optimally. Use the Click-iT reaction mix within 15 min of preparation.

Reagents	Volume (μL)
Deionized water	758
Reaction buffer (10×)	100
Copper Reagent	40
AbFluor 488 azide	2
10×Reducing Agent	100
Total volume	1000

2. Add 100 μL of Click-iT reaction mix to each sample and incubate the cells for 30 min at room temperature and protected from light.

3. Remove the reaction mix, then wash each well once with 0.1 mL of 1×BSA Wash Solution. Remove the solution.

4. Optional: Nuclear staining (500×DAPI stock solution mixed with PBS into 1× working solution) or antibody labeling.

Note: Keep the samples protected from light during incubations. If no additional staining is desired, proceed with imaging and analysis.

5. Analyze samples for green fluorescence generated by labeled DNA with Fluorescence Microscope (Ex/Em=501/525 nm). The nuclei were detected by Ex/Em = 360/460 nm.

Strawberry moment: In addition to detecting cell proliferation/toxicity based on DNA structure and cytoplasmic changes, in most cases, cell toxicity (Cat #: KTA1030) is evaluated based on the enzyme activity of LDH in the cell culture supernatant. In addition, more comprehensive monitoring of cell status needs to be analyzed from the perspective of apoptosis (Cat #: KTD102-EN), cell senescence (Cat #: KTA3030) and so on. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

Typical Data

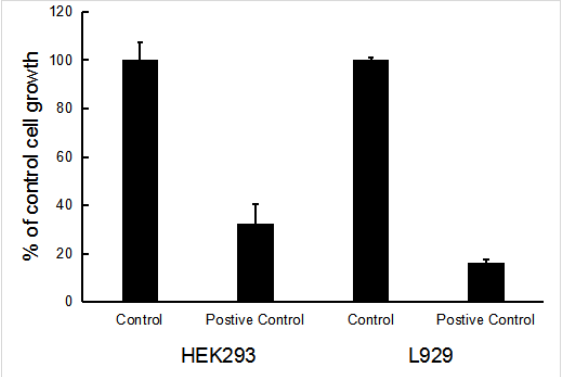

Figure 1. HEK293 cells and L929 cells were induced by this kit and were detected Cytotoxicity by CCK8 Assay.

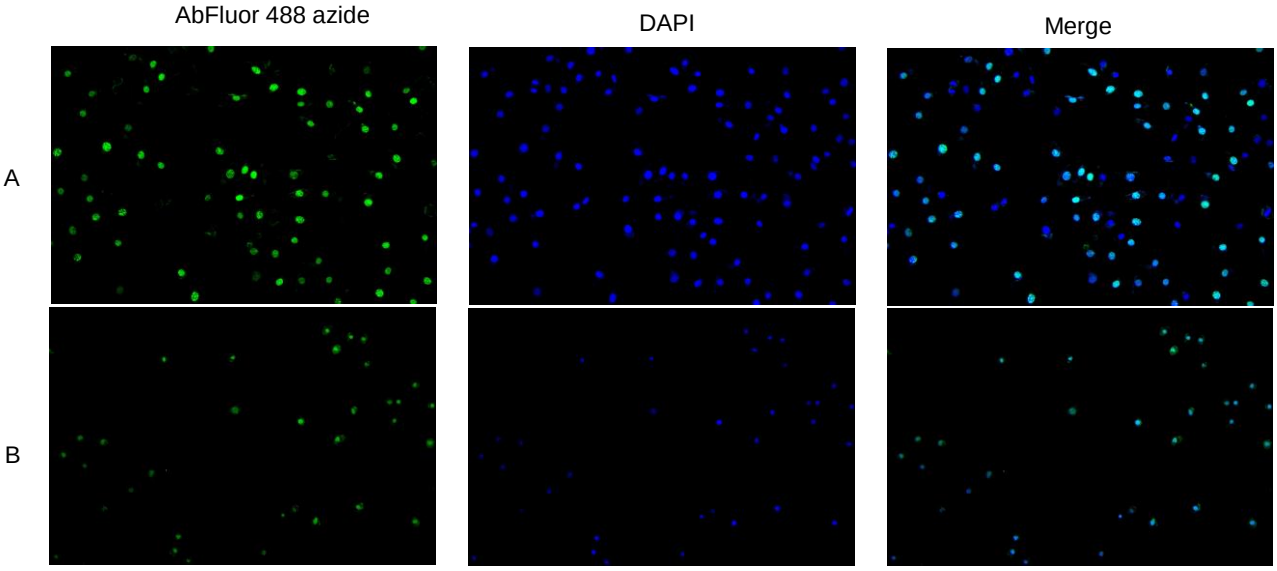

Figure 2. Hela cells were induced by this kit and were detected Cytotoxicity by EdU detection.

References

[1] Taylor, J. H., Woods, P. S., Hughes, W. L., (1957). The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidinee. Proc Natl Acad Sci U S A, 43(1), 122-128.

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[3] Shiwei Yang, Shanyu Gao, Tongming Liu, et al.Circular RNA SMARCA5 functions as an anti-tumor candidate in colon cancer by sponging microRNA-552. Cell Cycle, 2021 ;20(7):689-701.

[4] Angelamellisy Revelian Ndibalema, Deo Kabuye, Si Wen, et al. Empagliflozin Protects Against Proximal Renal Tubular Cell Injury Induced by High Glucose via Regulation of Hypoxia-Inducible Factor 1-Alpha.Diabetes Metab Syndr Obes, 2020;13:1953-1967.

[5] Mieczysława Irena Boguś, Anna Katarzyna Wrońska, Agata Kaczmarek, et al.In vitro screening of 65 mycotoxins for insecticidal potential. PLoS One, 2021;16(3):e0248772.

[6] Mengzhu Lv, Simeng Zhang, Yuqing Dong, et al. PolG Inhibits Gastric Cancer Glycolysis and Viability by Suppressing PKM2 Phosphorylation. Cancer Manag Res, 2021;13:1559-1570.

[7] Berna Kavakcıoğlu Yardımcı, Zehra Mollaoğlu. Antioxidant or pro-oxidant? The effects of boron compounds on Saccharomyces cerevisiae BY4741 strain. Prep Biochem Biotechnol, 2021;51(1):96-103.

Recommended Products

Catalog No	Product Name	Recommended reason
KTA1020	Cell Counting Kit-8 (CCK-8)	High sensitivity, reagent has extremely low cytotoxicity, and does not affect downstream experiments.
KTA1030	LDH Cytotoxicity Assay Kit	Easy to operate and evaluate cytotoxicity based on LDH markers, which are the most widely used in cytotoxicity studies.
KTA2031	Cell Proliferation EdU Image Kit (Orange Fluorescence)	No denaturation step, patented AbFluor dyes have good light stability and anti-quenching effect.
KTA2030	Cell Proliferation EdU Image Kit (Green Fluorescence)	No denaturation step, patented AbFluor dyes have good light stability and anti-quenching effect.
KTD102-EN	Apoptosis Assay Cocktail	Universal and standardized apoptosis detection product.
KTA3030	Senescence β-Galactosidase Staining Kit	Specific staining for senescent cells, suitable for aging detection of cultured cells and tissue slice samples.
KTA3022	Caspase-3 Assay Kit (Colorimetric)	The operation is convenient and fast, and it can detect apoptosis in early / middle stage.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.