WSE- 7610

EzCyto Proliferation Kit Instruction Manual

August 21, 2026 edition

1.Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

2.Purpose of use

This reagent is used to measure the number of viable cells and cellular metabolic activity using colorimetric and fluorescence methods with resazurin. Add 10 µL of this product to 100 µL of cell culture medium and measure the absorbance or fluorescence after a certain period of time.

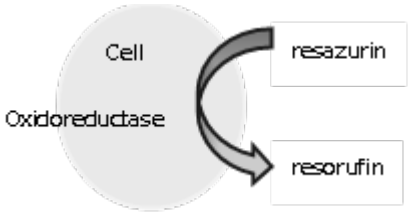
This product is suitable for the following uses:

- Cell viability measurement
- Cell proliferation measurement
- Cytotoxicity assays
- Anti-cancer drug screening
- IC 50 measurement
- Long-term monitoring of cultured cells

This method is non-destructive, and in most cases, cell culture can be continued after measurement.

3.Principle

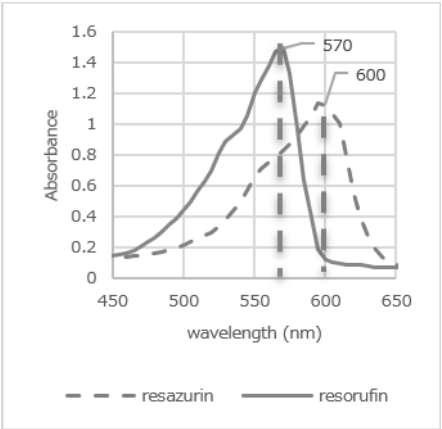
Resazurin, the main component of this reagent, is reduced to resorufin by oxidoreductases (such as dehydrogenases) in living cells. Cell count is determined by measuring the absorbance and fluorescence intensity changes that depend on this metabolic activity. In dead cells, this metabolic activity is stopped, so the reduction reaction does not proceed.



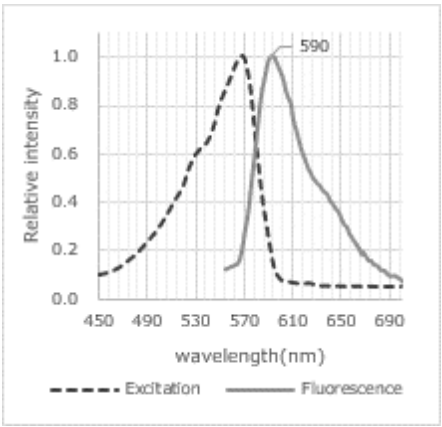
In colorimetric (absorbance) measurements, as the reduction reaction by living cells progresses, the absorption peak at 600 nm derived from unreacted resazurin decreases, and the absorption peak at 570 nm derived from the generated resorufin increases (a color change from blue to pink). The number of cells is quantified from the absorbance changes at these two wavelengths.

fluorescence measurements, the generated resorufins are strongly fluorescent substances. Irradiation with 560 nm excitation light produces fluorescence at 590 nm. Since this fluorescence intensity is proportional to the number of living cells, highly sensitive cell counting is possible.

Resazurin/resorufin absorbance spectrum



Resorufin fluorescence spectrum



4.Product Configuration

Name	Volume	Quantity
EzCyto Proliferation kit	25 mL	1 bottle

5.Composition

Name	Main component
EzCyto Proliferation kit	Buffer solution

This Product does not contain any poisonous or deleterious substances under the Poisonous and Deleterious Substances Control Law, or any substances subject to notification that exceed the exemption amounts stipulated under the Industrial Safety and Health Law or the PRTR Law. For details, please download the SDS for this product from the ATTO website (https://www.atto.co.jp/).

6.Storage

- This product will be transported refrigerated or frozen. After arrival, please store it frozen (-20 ℃ or below). It remains stable in an unopened state within the expiration date (1 year from the date of manufacture).
- After thawing, this product will remain stable for up to 6

months when stored in a light-shielded, refrigerated place (2-10°C).

7.Disposal method

Please dispose of this reagent in accordance with your institution's waste disposal procedures.

● Bottle material

Body: Polyethylene, Lid: Polypropylene

8.Items required other than this product

- CO_2 incubator
- 96-well plate
- Micropipette
- Absorbance plate reader
- Fluorescence plate reader

9.Precautions for use

- This product should be stored in a dark place, frozen (-20 ℃ or below).
- This product remains stable even after being frozen and thawed up to 5 times.
- This product is pre-prepared. No reagents need to be added, and no mixing or dilution is required.
- Before use, allow the solution to return to room temperature and mix thoroughly by inverting the container or similar method to ensure uniformity. Please be careful not to mix too vigorously, as this may cause foaming.
- Although this product is sterilized, please note that after opening, the sterilization state may not be maintained due to contamination by bacteria, etc.
  When measurement conditions include reducing substances such as ascorbic acid or DTT, this product will be reduced before it can react with cells. Please set up appropriate controls.
- All operations must be performed aseptically. Contamination with bacteria may react with this reagent and affect the measurement results.
- When dispensing samples or reagents into the measuring container, please be careful to avoid introducing air bubbles.
- If you continue cell culture after measurement, promptly change the culture medium. For adherent cells, remove the culture medium after the color reaction using an aspirator, then rinse several times with PBS or similar before replacing it with fresh medium.
  For suspension cells, after centrifugation, remove the supernatant, rinse several times with PBS or similar, and then replace with fresh culture medium.

10.How to use

(1) Measurement conditions

- Colorimetric measurement
  - Measurement wavelength: 570 nm
  - Reference wavelength: 600–620 nm
- Fluorescence measurement
  - Excitation wavelength: 560–560 nm
  - Fluorescence wavelength: 590–610 nm

(2) Method for seeding **cultured cells**

The target of measurement is **cultured cells,** collect cells in the logarithmic growth phase and seed them in a 96-well plate with 100 µL of cell culture medium per well. The approximate number of cells is as follows. Prepare a blank well (Blank1) containing only the culture medium. To check the effect of the test substance on cells, after seeding the cells and culturing them for a certain period *, add the test substance to the culture medium as appropriate (Sample) . At that time, also prepare a well (Blank2) containing only the test substance and culture medium, and a well (Control) containing only cells without the test substance.

*For adherent cells, continue until they adhere to the well.This step can be omitted for floating cells.

Adherent cells: 300-30,000 cells / well
Suspension cells: 500-50,000 cells /well

Blank 1	Wells containing only culture medium
Blank2	Wells containing only the test substance and culture medium.
Control	Wells containing only cells
Sample	wells in which the test substance was added

(3) Method of **bacterial** inoculation

Measuring **bacteria,** collect bacteria in the logarithmic growth phase and inoculate 100 µL per well into a 96-well plate. The approximate bacterial count is 10^5 ~10^7 This will be CFU/mL. Prepare blank wells containing only culture medium. To check the effect of the test substance, after inoculating the bacteria and culturing for a certain period of time, add 1/10 the amount of the test substance to the bacterial suspension. At that time, prepare wells containing only the test substance and culture medium, as well as wells without the test substance .

(4) Measurement method

(2) or (3) is incubated for a certain period of time, and then 1/10 the volume of this product is added to the culture medium. For a 96-well plate, add 10 µL to 100 µL of culture medium and mix gently.

* The amount of reagent required per 96-well plate is 1 to 1.2 mL.
* Please allow this product to return to room temperature beforehand.
* All operations must be performed aseptically. Reactions with contaminants may affect the measurement results.
* When adding this product, please be careful to avoid introducing air bubbles.

(4) -1 Incubation

For cultured cells, culture at 37 °C under 5% CO_2 for 1 to 4 hours. The optimal culture time varies depending on the cell type and cell density. Please refer to the guidelines below.

* When **bacteria** are the target of measurement, they will be cultured at their optimal temperature.
* In cases of high cell density (high bacterial count) or with highly metabolically active cells or bacterial species, a signal may be observed immediately after addition.

Estimated incubation time

cultured cells

Hela	1-2 hours
HEK293	1-2 hours
HepG2	1-3 hours
K562	2-3 hours
KYHG-1	3-4 hours

This is a guideline based on seeding at 1 x 10^4 cells/ well .

bacteria

Escherichia coli	2-4 hours

Staphylococcus aureus 1-3 hours
Enterococcus faecalis 1-3 hours
Pseudomonas aeruginosa 2-4 hours
This is an estimate based on vaccination at $1x10^6$ CFU /well.

(4) -2. Absorbance or fluorescence measurement
The microplate is placed in a plate reader capable of absorbance or fluorescence measurement, and the measurement is performed.
For absorbance measurements, the measurement is performed at 570 nm, with a reference wavelength of 600–620 nm.
For fluorescence measurements, set the excitation wavelength to 560 nm and the fluorescence wavelength to 590 nm, and then perform the measurement.
* Microplates that have been kept in a cool, dark place to prevent evaporation and refrigeration can be stored for several days, although the values will gradually increase .

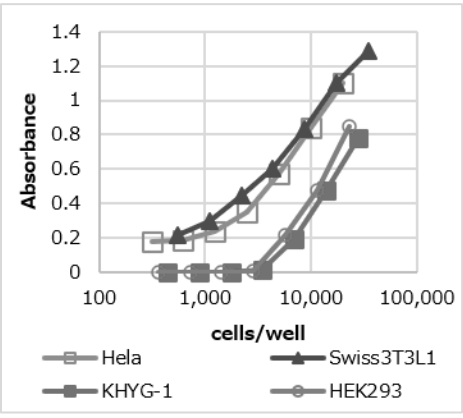
(5) Data Analysis
In the case of absorbance measurements, a blanking process is performed after normalization using the reference wavelength.
a. Normalization by reference wavelength
Subtract the measurement value at 600 nm from the measurement value at 570 nm.
b. Blanking process
Subtract the measurements from the blank well (Blank 1 or 2) from all data normalized by the reference wavelength.
For fluorescence measurements, subtract the measurement from the blank well (Blank 1 or 2) from all the data.

Cell viability is calculated using the following formula:

Cell viability (%)
= [(sample – Blank 2) / (Control - Blank 1)] x 100

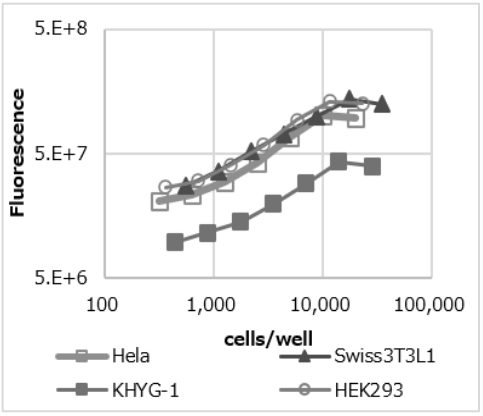
11.Measurement data

Cell density-dependent absorbance measurement



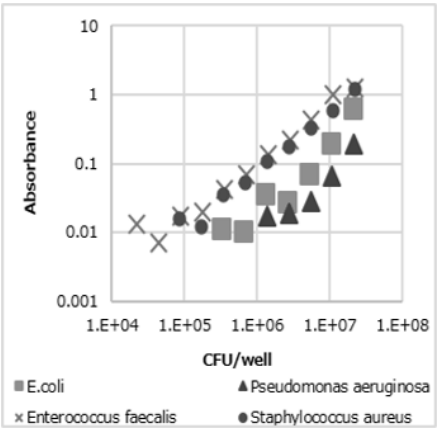
A 1/2 dilution series of cell suspensions was prepared in culture medium and incubated for a certain period of time. After adding the product, measurements were taken at 570 and 600 nm after 2 hours. Cellular metabolic activity was evaluated as the absorbance at 570 nm minus the absorbance at 600 nm (A570-A600). The results showed that in all cell lines, absorbance increased with increasing seeded cell numbers, confirming that the product exhibits a signal corresponding to the number of viable cells. However, differences in absorbance were observed between cell lines even with the same number of cells.

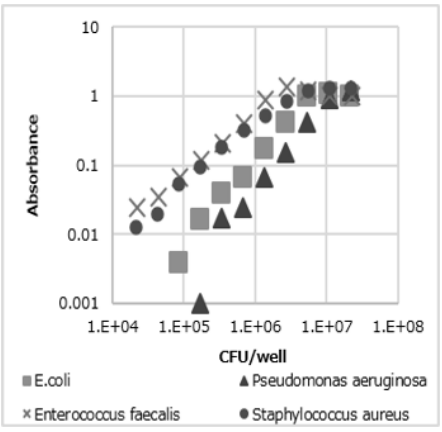
Cell density-dependent fluorescence measurement



Fluorescence measurements using the same microplate as the " cell density-dependent absorbance measurement " described above are shown. This demonstrates that cell lines that could not be detected by absorbance measurements at low densities can be detected by fluorescence measurements.

Bacterial count-dependent absorbance measurement

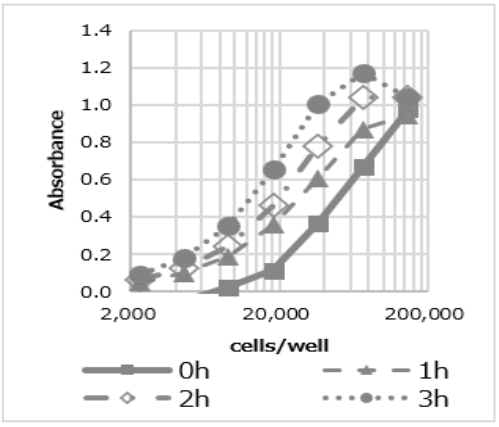




Using bacteria in the logarithmic growth phase, a 1/2 dilution series was prepared in Mueller-Hinton medium and cultured for a set period of time. After adding the product, measurements were taken at 570 and 600 nm after 1 hour (upper graph) and 3 hours (lower graph). The value obtained by subtracting the absorbance at 600 nm from the absorbance at 570 nm (A570-A600) was used for evaluation. After 1 hour, E. Faecalis could

be detected at 10^4 CFU/well and S. aureus at 10^5 CFU /well, but no difference in bacterial count was observed for E. coli and P. aeruginosa below 10^6 CFU /mL. After 3 hours, all were detectable up to 10^5 CFU /well. On the other hand, absorbance saturation was confirmed above 10^6 CFU / well.

Change in absorbance due to reaction time



A 1/2 dilution series of HeLa cells was prepared in DMEM medium and cultured for a certain period of time. After adding this product, absorbance measurements were taken at regular intervals starting immediately after addition. Under conditions of high cell density, a signal was observed immediately after addition. On the other hand, after 3 hours, a plateau was observed under conditions of high cell density.

troubleshooting

Symptoms	Causes	Solution
No color development (no fluorescence detected)	Low number of cells	Please increase the number of cells seeded.
	Low cell survival rate	Check the condition of the cells and use fresh, logarithmic growth-phase cells.
	Short reaction time	Please extend the incubation period.
	Degradation and improper storage of reagents	Please check the expiration date and storage conditions and use fresh reagents.
	Plate reader configuration error	Please check the recommended wavelengths (absorbance: 570 nm / reference 600 nm, fluorescence: Ex 530–560 nm, Em around 590 nm).
The color payoff is very weak.	Low metabolic activity of cells	Check the cell type and culture conditions and extend the reaction time.
	Low number of cells	Please increase the seeding density.
	Short reaction time	Please extend the incubation period.
	The culture temperature and CO_2 conditions are inappropriate.	Please carry out the reaction under appropriate culture conditions such as 37 °C and 5% CO_2.
The colors are very vibrant.	There are too many cells.	Please reduce the number of cells seeded.
	The reaction time is too long.	Please shorten the incubation period.
	The signal is outside the measurement range.	Optimize the measurement conditions and adjust the cell count as needed.
All wells will have the same value.	The reaction is saturated.	Reduce the number of cells or the reaction time.
	Plate reader settings are incorrect.	Please check the recommended wavelengths (absorbance: 570 nm / reference 600 nm, fluorescence: Ex 530–560 nm, Em around 590 nm).
High background	Blank correction has not been performed.	Please set either a blank sample containing only reagents or a blank sample containing culture medium and reagents.
	Influence of culture medium components	Measure the culture medium blank, subtract the background value, and then perform the analysis.
	The reagents are degrading due to light.	Store reagents in a light-shielded place and avoid prolonged exposure to light during use.
The drug-treated group showed a higher signal than expected.	The drug is directly reducing resazurin.	Set up a blank sample containing only the drug (without cells) and observe the effects.
	Effect of reducing agents (DTT, β-mercaptoethanol, ascorbic acid, etc.)	Under conditions involving reducing substances, please set up appropriate controls.
Cytotoxicity cannot be detected.	Low drug concentration	Please review the drug concentration.
	Short processing time with the drug	Depending on the cell type, extend the treatment time with the drug.
	The timing of resazurin addition is inappropriate.	Add the reagent at the appropriate time after chemical treatment.
There is a large variation between wells.	Cell seeding is not uniform	Thoroughly mix the cell suspension and sow the seeds evenly.
	Pipetting error	Use a calibrated pipette and dispense accurately.
	There is a difference in the reagent addition time.	Add the solution quickly using a multichannel pipette or similar device.
The signals on the outer edge of the plate are different.	Edge effect	It is recommended to either fill the outer wells with PBS or culture medium, or to exclude them from the analysis.
Low reproducibility from experiment to experiment	Different number of passages	Please use cells that have undergone the same number of passages.
	Cell density is not constant	Seed the cells under the same conditions.
	Culture conditions	Please keep the culture

	are not consistent.	medium, incubation time, temperature, and CO_2 concentration constant.
The signal continues to increase over time.	Resolphin production is continuing.	Please take the measurement within the recommended reaction time. Avoid prolonged reaction times.
The signal decreases midway through.	Resolphins are further reduced.	Reduce the reaction time and set the optimal measurement timing.
The signal is weak in the primary cultured cells.	Low metabolic activity of cells	Increase the number of cells or extend the reaction time.
Low reproducibility in suspension cells	The cells are settling.	Mix the cell suspension thoroughly before adding the reagents.
Fluorescence measurement and absorbance measurement yield different results.	Differences in measurement sensitivity	For identical experiments, please compare results using the same measurement method. Fluorescence measurements are generally highly sensitive.

12.Reference material

Even with the same protocol, slight differences in technique can significantly alter experimental results. "Tips and tricks" are crucial for obtaining optimal results. You can download various "experimental tips" from the ATTO website; please take a look.

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