bioGenous LivingCell-Fluo™ Organoid Vitality Assay Kit

Catalog：E238004

Product Description

bioGenous LivingCell-Fluo™ Organoid Vitality Assay Kit (LivingCell-Fluo™) offers a fluorescent detection method for analyzing cell and organoid vitality in multi-well plates. The assay does not require cell lysis: the fluorescent dye permeates live cells and interacts with metabolic reductase enzymes within them to produce a fluorescent signal. To ensure accurate vitality assessment during the assay, bioGenous LivingCell-Fluo™ Organoid Vitality Assay Kit (serum-free) includes a live cell fluorescent dye and a specialized buffer for organoid vitality analysis. After preparing the detection solution, it is added to the cell/organoid culture system to measure the fluorescent signal. This assay evaluates cell vitality by measuring metabolic activity: live cells can reduce the fluorescent dye in the kit to a red fluorescent product (excitation wavelength: 560 nm; emission wavelength: 590 nm), while non-viable cells quickly lose metabolic activity and thus cannot reduce the dye, resulting in no fluorescence signal. The intensity of the signal is directly proportional to the number of viable cells in the system. Additionally, the kit can be used in conjunction with bioGenous™ LivingCell Vitality Analysis System for straightforward and rapid evaluation of organoid vitality.

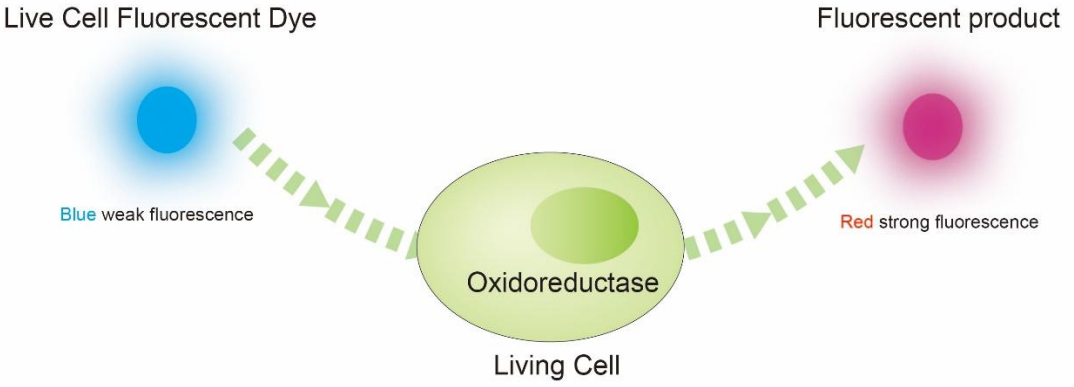


Figure 1. Schematic diagram of detection principle

Product Features

bioGenous LivingCell-Fluo™ Organoid Vitality Assay Kit offers several key advantages:
(a) **Non-Digestion, Non-Lysis Required**; (b) **Highly Sensitive Linear Response**: The assay provides a highly sensitive linear response with a minimum detection time of 30 minutes, allowing for the assessment of at least 100 cells per well. (c) **Convenient Addition:** No washing required before or after detection; (d) **Non-Toxic, Non-Sterile Fluorescent Dye**: The fluorescent dye used is non-toxic and does not interfere with normal cell metabolism or gene expression. (e) **Fluorescence-Based Detection**: The assay operates on a fluorescence-based principle, eliminating the need for black or opaque-bottom cell culture plates. (f) **Organoid-Specific Vitality Detection Buffer**; (g) **Calibration and Error Correction**: The assay allows for multiple detections to calibrate and correct data deviations caused by manual plate handling errors during the experiment.

Product Information

Sealed and protect from light; Stored 4°C for up to 1 year, stable when stored at -20°C for 2 years. Transport with ice packs.

Component	Cat#	Volume	Storage& Stability
bioGenous LivingCell-Fluo™ Organoid Vitality Assay Kit	E238004	10 mL/50 mL （1000/5000 Tests）	4 °C, 1 year -20°C, 2 years
Component A: Live Cell Fluorescent Dye (10X)	-	10 mL/50 mL	4 °C, 1 year -20°C, 2 years
Component B: Organoid Vitality Detection Buffer	-	100 mL/500 mL	4°C, 1 year -20 °C, 2 years

Protocol of bioGenous LivingCell-Fluo™ Organoid Vitality Assay

Additional Equipment and Reagent required

Single-channel or multi-channel pipettes, 3D micro-tissue culture multi-well plates, a fluorescent microplate reader, or bioGenous™ LivingCell Vitality Analysis System.

Preparation of Assay Reagents

1. Preparation of 1× Organoid Vitality Assay Working Solution:
   Dilute Component A (Live Cell Fluorescent Dye, 10×) with Component B (Organoid Vitality Detection Buffer) to prepare the 1× Organoid Vitality Assay Working Solution. To prepare 10 mL Working Solution, mix 9 mL of Component B with 1 mL of Component A. Mix thoroughly and use within 120 min.
   ***Note**: For the first use of the product, it is recommended to aliquot at 3-5 times the amount needed for each use. Component A contains fluorescent dye, stored protected from light after aliquoting.*
2. Gently invert the solution a few times to mix the assay working solution before use.
3. **For 2D Cell Viability Assays**: Dilute the live cell fluorescent dye (Component A) in complete cell culture medium. It is recommended to use a 96-well plate for detection. For other types of plates, refer to the following table for the required volume of working solution and component quantities.

Plate	Volume of 1X Organoid Vitality Assay Working Solution (μL)	Volume of Component A (μL)	Volume of Component B/Complete Culture Medium (μL)
6-well plate	2000	200	1800
12-well plate	1000	100	900
24-well plate	500	50	450
48-well plate	200	20	180
96-well plate	100	10	90

384-well plate	40	4	36

Continuous vitality assessment of organoids

Example workflow for continuous Organoid Viability Assessment (96-well plate):

1. Remove the 96-well cell culture plate from the incubator. Discard the existing complete culture medium from the wells as thoroughly as possible to avoid: (a) further dilution of the dye, (b) residual substances from the organoid culture affecting the dye, and (c) interference from components in the complete organoid culture medium.
2. Add 100 µL of 1X Organoid Vitality Assay Working Solution slowly along the side walls of each well. Set wells without organoids as dye-negative controls. Incubate the plate in the incubator for 30-120 min. When the working solution in the wells with cells or organoids changes color from blue to pink, the next step can be performed.
   ***Note**: If the cell quantity is low, the working solution may remain blue after 30-120 min of incubation, which does not affect the detection results.*
3. Fluorescence Measurement: use a fluorescence microplate reader (Excitation (Ex)/ Emission (Em) wavelengths: **560(±10)$_{Ex}$/590(±10)$_{Em}$**) or bioGenous™ LivingCell Vitality Analysis System to measure and record the total fluorescence intensity.
   ***Note**: ±10 indicates the detection range of ±10 nm around the Ex (560 nm) / Em (590 nm) wavelengths.*
4. After measurement, discard the liquid from the 96-well plate and replace it with fresh complete culture medium. Continue incubating for 2 days.
5. Repeat steps 1, 2, and 3. Normally, the vitality of organoid cells in healthy growth conditions should increase with longer culture time. For example, the total fluorescence value should increase by more than 20% every 2 days.
6. Data Analysis: Analyze the relative vitality of the organoids based on the total fluorescence intensity (subtracting the dye negative control).

Organoid drug sensitivity assay

For Organoid Drug Sensitivity Assay, the experimental procedure is as follows (96-well plate):

1. Digest organoids to single cells for passaging. For example, seed 3000 cells per well with 3-5 µL of matrigel in a 96-well plate and culture for 2 days to allow single cells to re-form organoids. Before drug treatment, LivingCell-Fluo™ can be used to measure the viability of organoids in each well to calibrate plating errors **(optional)**:
   a. Remove the 96-well plate from the incubator and discard the existing complete medium from the wells.
   b. Slowly add 100 µL of 1× Organoid Vitality Assay Working Solution to each well along the sidewalls. Set wells without organoids as dye-negative controls. Incubate the plate in the incubator for 30-120 min. If the working solution color with cells or organoids changes from blue to pink (***note**: If the cell quantity is low, the working solution may remain blue after 30-120 min of incubation, which does not affect the detection results*), proceed to the next step.
   c. Measure the total fluorescence intensity using a fluorescence microplate reader (Ex/Em: 560(±10)$_{Ex}$/590(±10)$_{Em}$ or bioGenous™ LivingCell Vitality Analysis System. Record the total fluorescence intensity.

2. Discard the liquid from the 96-well plate and add drugs at different concentrations (prepare drug solutions in organoid complete medium, including drug negative controls, with three or more replicates per group). Continue incubation. After 3-5 days of drug treatment, assess organoid vitality using the LivingCell-Fluo™ assay:
3. Follow the same procedure as in step 1: a), b), and c), ensuring that the incubation times before and after drug treatment are consistent.
4. Analyze the relative vitality of organoids based on the total fluorescence intensity (subtracting the values of dye and drug negative controls).
5. The formula for calculating the relative vitality of organoids in drug sensitivity assays, accounting for seeding errors, is as follows:

$$Cell\ Viability\ (of\ Control, \%) = \frac{\frac{(Ox_{A2} - \overline{NC_{A2}})}{(Ox_{A1} - \overline{NC_{A1}})}}{\sum_{i=0}^{n}\left[\frac{(VC_{A2} - \overline{NC_{A2}})}{(VC_{A1} - \overline{NC_{A1}})}\right]/n} \times 100\%$$

n (n ≥ 3)：the number of replicates in the drug sensitivity assay；A1：measurements taken before drug treatment；A2：measurements taken after drug treatment；Ox：different drug concentration treatment groups；NC：dye negative control group；VC：drug negative control group；$\overline{NC_{A2}}$：the average value of the dye negative control group after drug treatment；$\overline{NC_{A1}}$：the average value of the dye negative control group.

6. If LivingCell-Fluo™ is used only after drug treatment, the organoid vitality calculation formula is:

$$Cell\ Viability\ (of\ Control, \%) = \frac{Ox - \overline{NC}}{\overline{VC} - \overline{NC}} \times 100\%$$

Ox: different drug concentration treatment groups; NC: dye negative control group; VC: drug negative control group；$\overline{NC}$: the average value of the dye negative control group; $\overline{VC}$: the average value of the drug negative control group.

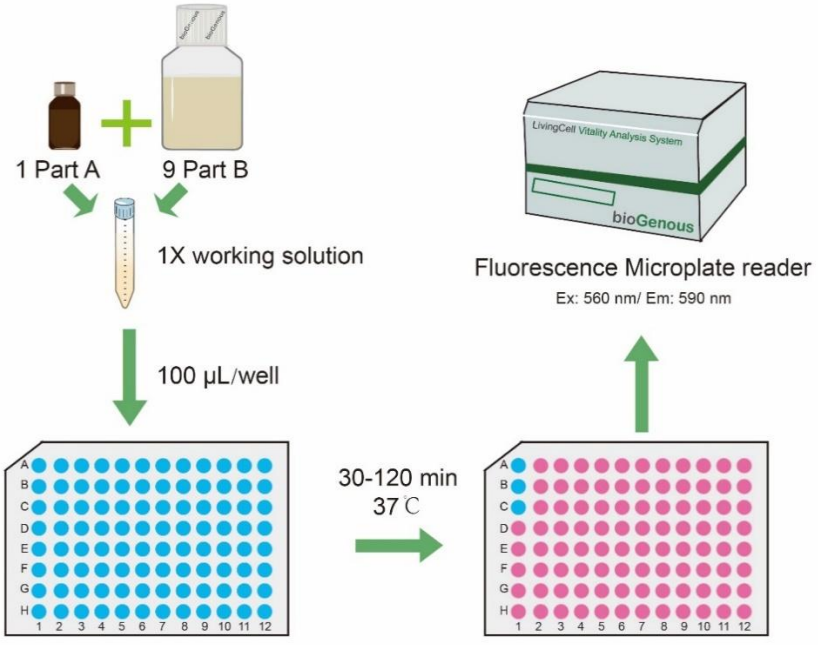


Figure 2. Schematic diagram of experimental operation process

Precautions

1. Proper cell density can enhance assay sensitivity. For a 96-well plate, it is recommended to seed more than 100 and fewer than 100,000 cells per well.
2. The entire assay should be conducted away from direct sunlight (laboratory white light has minimal impact) and under sterile conditions, as microbial contamination can affect the results.
3. Incubation time after adding the assay reagent is dependent on cell concentration. For low cell concentrations, extend the incubation time, and for high cell concentrations, shorten the incubation time. It is recommended to use a fluorescence microplate reader for detection from the bottom of the plate, as condensation on the plate lid and marker pen marks above the wells will significantly affect the readings.
4. When calculating vitality values, subtract the values of negative controls (wells without cells/organoids). For drug sensitivity assays, subtract the values of drug controls.

Applications

1. Analyze the vitality of Lung Adenocarcinoma organoids (LAC) with varying cell numbers using LivingCell-Fluo™ Cell Vitality is shown in Figure 3A.
2. Perform continuous growth monitoring of LAC and Human Intestinal organoids (HIO) using LivingCell-Fluo™ Cell Vitality. Typically, the cell vitality of normally growing organoids should increase with time. For example, the total fluorescence value should increase by more than 20% every 2 days, as shown in Figure 3B.
3. Conduct drug sensitivity assays on Breast Cancer organoids to paclitaxel using LivingCell-Fluo™ and bioGenous™ ATP Assay Kit (Catalog No. E238003). The drug sensitivity curve is shown in Figure 3C. LivingCell-Fluo™ demonstrates results comparable to the ATP assay (comparing IC50 and drug sensitivity curve fitting). (Figure 3C)
4. Assess the 3D cell vitality of different numbers of CHO cell lines seeded in Matrigel using LivingCell-Fluo™ Cell Vitality. Cell vitality is illustrated in Figure 3D, showing the high sensitivity and linear response of LivingCell-Fluo™ Cell Vitality. (Figure 3D)
5. Compare the vitality of 3D HIO with different cell numbers using LivingCell-Fluo™ Cell Vitality and CCK-8 (same experimental parameters). Results indicate that CCK-8 does not show a linear relationship with cell numbers (50-300), and at 250 cells, absorbance values decrease despite increased cell numbers, suggesting lower sensitivity and stability of CCK-8. In contrast, LivingCell-Fluo™ Cell Vitality shows a good linear relationship between cell number and fluorescence signal, demonstrating higher sensitivity and stability compared to CCK-8 (Figure 4).
6. Compare the vitality of 2D cells (293T and 3T3 cell lines) using LivingCell-Fluo™ Cell Vitality and CCK-8 (same experimental parameters). Results show that CCK-8 does not exhibit a linear relationship with cell numbers (50-2000), and at 2000 cells, absorbance values decrease despite increased cell numbers. LivingCell-Fluo™ Cell Vitality provides a good linear relationship between cell number and fluorescence intensity, showing higher sensitivity and stability compared to CCK-8 (Figure 4).

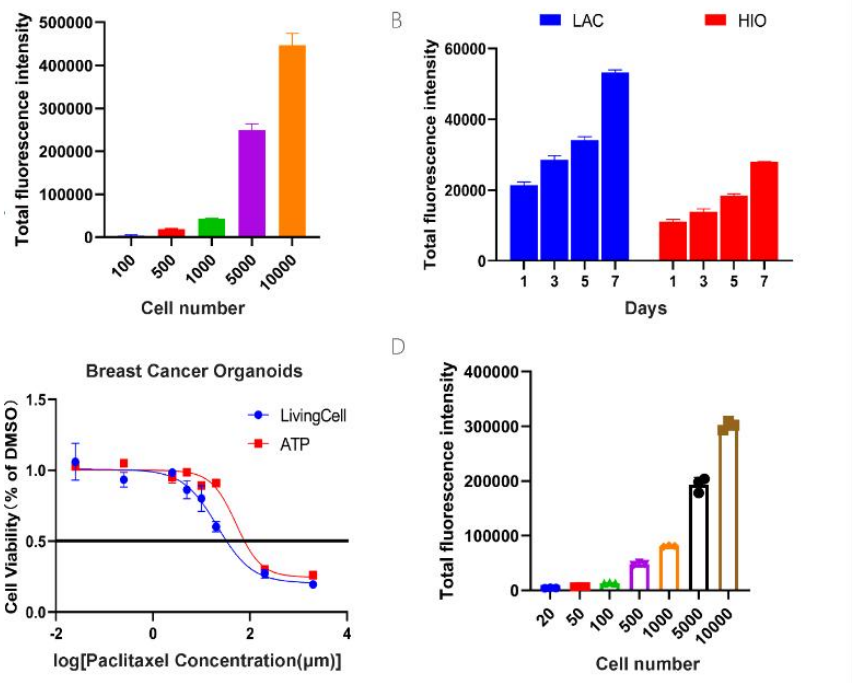
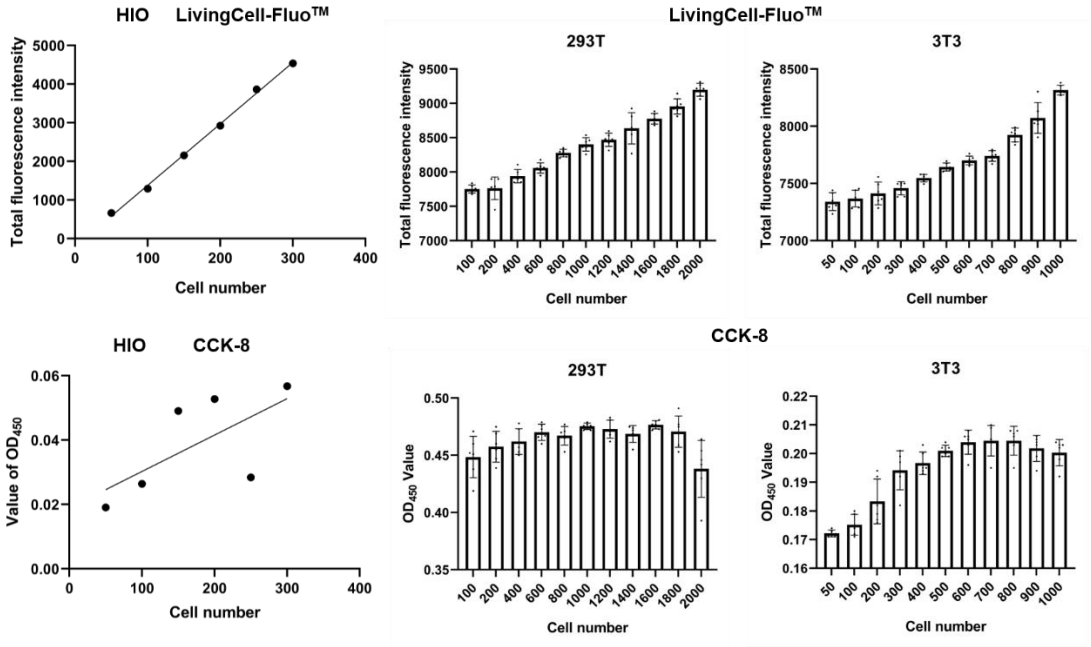


Figure 3: LivingCell Fluo™ Cell Vitality detection of 3D cell



viability

Figure 4 Comparison of LivingCell Fluo™ Cell Vitality and CCK8 for detecting the vitality of 3D HIOs and 2D cell lines (293T and 3T3)

Quality Control

All components are negative for bacterial and fungal contamination. Certificate of authenticity (COAs) for all other products are available upon request.

Safety information

Read the Safety Data Sheets (SDSs) and follow the manufacture's instruction.

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Contact and Support

For questions, suggestions, and technical supports, please contact us at E-mail: info@biogenous.cn.

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