**Experiment time: 2025-03-10, 18:00 - 2025-03-17, 21:00**

# Growth Curve Measurement (I)

## I. Objective of the experiment

The proliferative capacity of the T24 cell line and the highly drug-resistant T24-RC48 cell line under 200μg/ml RC48 treatment was assessed using the CCK-8 assay over a 72-hour period. Growth curves were plotted to evaluate the drug's inhibitory effects on the growth of the different cell lines. The conditions for formal growth curve measurement were explored.

## II. Experimental content

### 2.1 Experimental design

Cell types: T24 wild-type cell line and highly drug-resistant T24-RC48 cell line.

Drug treatment: Vedotin (RC48) was administered at a concentration of 200 μg/ml.

Time: 0h,24h,48h,72h.

Number of replicates: 6 wells per group.

### 2.2 Measurement principle

WST-8 in CCK-8 reagent was reduced to an orange-colored formazan product by mitochondrial dehydrogenase of cells, and its absorbance (OD=450nm) was positively correlated with the number of living cells. The absorbance is directly proportional to the number of viable cells, allowing for the assessment of cell viability.

## III. Materials and reagents

### 3.1 Materials

The 1000μl and 100μl pipette tips and 15ml centrifuge tubes were purchased from Axygen

96-well plates (Corning Inc.) x 4

Micropipettes: Eppendorf

T-25 cell culture flasks were purchased from Corning

Human bladder transitional cell carcinoma cell line (T24)

Highly drug-resistant cell line T24-RC48

### 3.2 Reagents

PBS and trypsin were purchased from Gibco, USA

McCoy's 5A medium from Shanghai Yuanpei Biotechnology Co., Ltd., China

Fetal bovine serum (FBS, Gibco)

Disitamab vedotin (RC48, Rongchang Biopharmaceutical Co., LTD.)

CCK-8 kit

**IV. Experimental instruments**

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

Microplate reader

**V. Experimental steps**

5.1 T24 and highly drug-resistant T24-RC48 cell lines in the logarithmic growth phase were harvested, digested with trypsin, centrifuged, and resuspended. The cell density was adjusted to $4 \times 10^4$ cells/mL.

5.2 The cell suspension was inoculated into four 96-well plates according to the experimental design, with each plate receiving 12 wells per cell type. Each well contained 100μl of culture medium (containing $4\times10^3$ cells), and PBS was added to the edge wells to prevent evaporation. The plates were then cultured in an incubator maintained at 37°C with 5% $CO_2$ concentration.

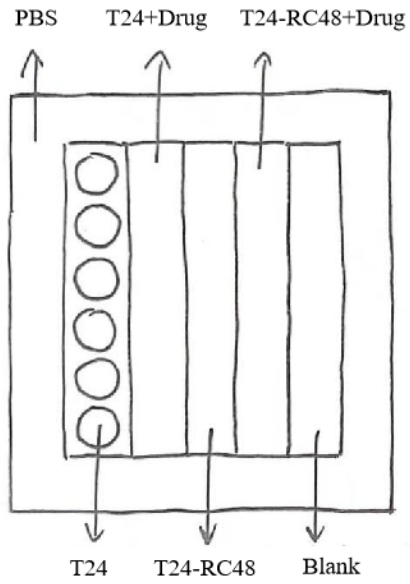


Figure 1 Distribution of cell suspensions in each group in the 96-well plate

5.3 After overnight cell seeding, aspirate the old medium and replace with fresh medium in three 96-well plates (add 6 wells per plate with fresh medium containing 200μg/ml RC48). Set the time to 0 and incubate at 37°C with 5% $CO_2$. For the remaining plate (Plate 1), add the chromogenic solution to the culture wells according to the CCK-8 kit instructions. Incubate for 1 hour and measure the absorbance at 450nm using a microplate reader.

5.4 At each time point (24, 48, and 72 hours), the corresponding 96-well plate was taken out from the incubator. Treat it according to the instructions of CCK-8 kit and measure the absorbance value at 450nm.

5.5 The time of treatment with RC48 was taken as the horizontal coordinate, and the OD value of the experimental group at 450nm was taken as the vertical coordinate with the OD value of the blank control hole. The time-OD value curve, namely the cell proliferation curve, was plotted.

## VI. Experimental results

6.1 Cell morphology observation

As can be seen from the inverted phase contrast microscope photo in Figure 2, after 72 hours of treatment, T24-RC48 cells still maintain a relatively good growth state, the cell morphology is relatively intact, and the cells reached approximately 100% confluence.

6.2 Changes in cell proliferation capacity

In the absence of RC48 treatment, both cell lines exhibited stable proliferation trends with no significant difference in doubling time, indicating comparable growth patterns under normal culture conditions. When treated with 200μg/ml RC48, the T24-RC48 cell line showed a slight increase in doubling time. However, this extension remained significantly smaller compared to the parent T24 cells post-treatment and notably shorter than the extended duration observed in the drug-treated T24 cells. The proliferation curve of T24 cells treated with RC48 was notably lower than pre-treatment levels, demonstrating a clear inhibitory trend.

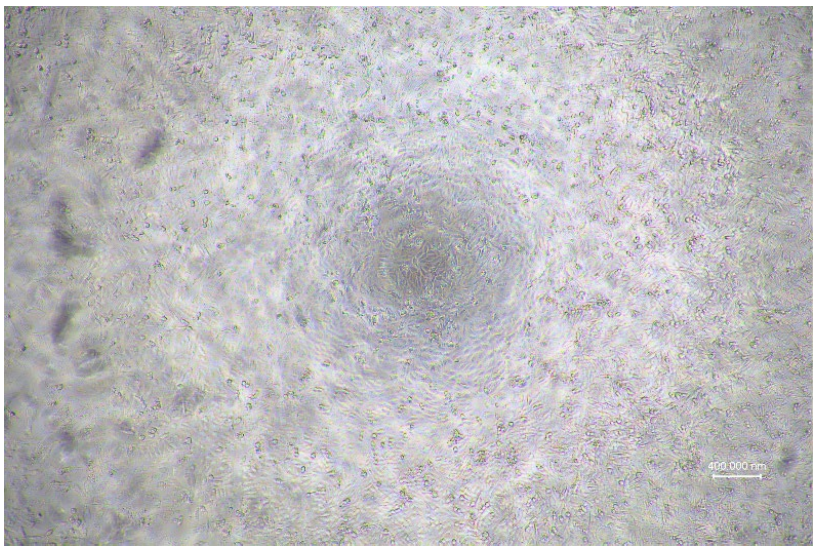

Figure 2 Photo of T24-RC48 cells after 72h under inverted phase microscope

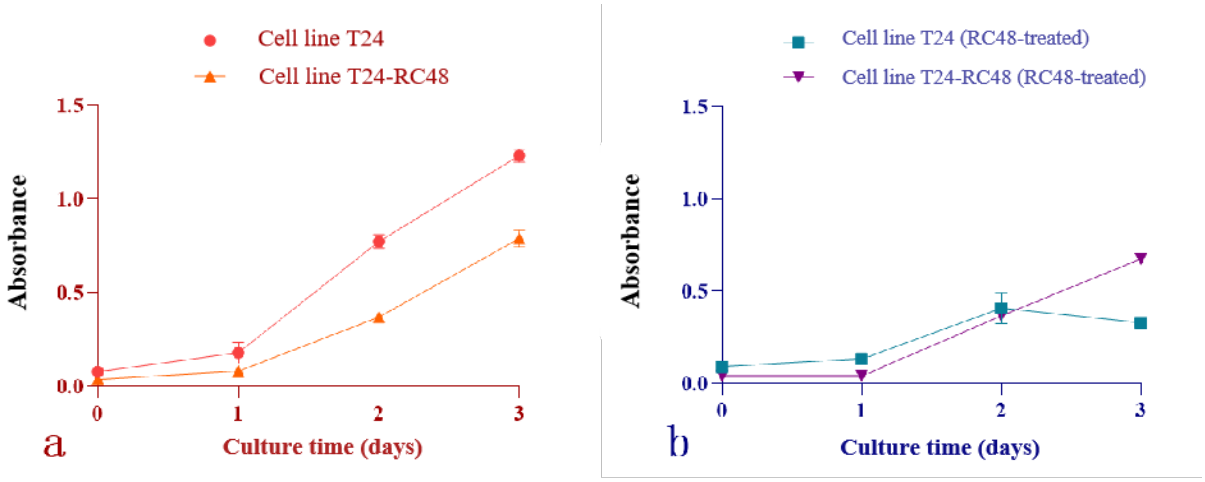


Figure 3 Growth curves of T24 and T24-RC48 cells before (3a) and after (3b) drug addition

**VII. Results analysis**

After 72 hours of culture, the morphology of cells without medication was relatively intact, and the cell density was close to 100%, indicating that in this experiment, the initial cell seeding density and the duration of the assay were appropriate, which provided a reference for the subsequent 5-day growth curve measurement, suggesting that we should appropriately reduce the plate concentration.

As shown in Figure 3, the doubling time of the drug-resistant T24-RC48 cell line showed no significant difference from its parent cell T24. After drug treatment, the doubling time of T24-RC48 did not show a marked extension compared to untreated cells and was significantly shorter than that of the parent T24 cells treated with the drug. This indicates that the T24-RC48 cell line cultured in this study demonstrates stronger drug resistance to RC48 compared to traditional T24 cell lines. Although drug treatment affected the growth rate of T24-RC48 cells, the impact was much smaller than that of RC48 on T24 cells. This may be because T24-RC48 cells have gradually developed adaptive mechanisms against RC48 drugs through long-term culture and screening, such as enhancing drug-metabolizing enzyme activity, increasing efflux pump expression, and altering intracellular signaling pathways to reduce the inhibitory effect of drugs on cell proliferation. Meanwhile, the proliferation curve of T24 cells treated with the drug showed a clear inhibitory trend, suggesting that the 200μg/ml drug concentration is reasonable and can be adopted in subsequent experiments.

This finding holds significant implications for investigating the drug resistance mechanisms of T24 cells to RC48. It validates the experimental hypothesis that T24-RC48 cells maintain relatively high proliferative capacity under RC48 treatment, establishing a foundation for further exploration of resistance mechanisms. The results suggest that subsequent studies should focus on elucidating the specific resistance mechanisms in T24-RC48 cells, which could provide theoretical foundations for developing more effective anticancer drugs and therapeutic strategies.