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🌐 Blasticidin titration of cancer cell lines

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Cellular Generation and Phenotyping

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ABSTRACT

This protocol is used to identify the optimum blasticidin concentration for the selection of Cas9 positive cancer cell lines.

Process diagram:

GUIDELINES

- Ensure the cell suspension is mixed thoroughly to create an even single cell suspension before plating.
- All steps involved in the plate set up, including seeding cells, media, antibiotics and CellTiter-Glo should be carried out using reservoirs and multi-channel pipettes where possible to avoid ergonomic strain and to maintain homogenous solutions throughout.
- It is essential to use black 96-well plates in this protocol, as luminescence can carry over into neighbouring wells in clear plates.

MATERIALS

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⊗ CellTiter-Glo(R) 2.0 Assay **Promega Catalog #G9241**

⊗ Falcon™ 15mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-959-53A**

⊗ TrypLE™ Express Enzyme (1X), no phenol red **Thermo Fisher Catalog #12604021**

⊗ Reagent Reservoir **Thermo Fisher Catalog #9510047**

⊗ DPBS **Invitrogen - Thermo Fisher Catalog #14190**

⊗ 10mg/ml Blasticidin **InvivoGen Catalog #ant-bl-1**

⊗ Black walled 96 well plate **Fisher Scientific Catalog #10419822**

Select an appropriate culture media for your cell line. Common culture medias used for cancer cell lines are serum supplemented Advanced DMEM F-12 or RPMI, in the presence of pen-strep.

Equipment

Light Microscope

Microbiology safety cabinet (MSC)

Pipette Boy

Stripettes

Pipettes and tips

Centrifuge

Multichannel Pipette and tips

🔥 37 °C , 5%CO₂ incubator

Plate reader

SAFETY WARNINGS

- Blasticidin is toxic if swallowed and harmful in contact with skin.
- CellTiter-Glo is harmful to aquatic life with long lasting effects.

BEFORE START INSTRUCTIONS

- Pre-warm culture media to room-temperature.
- Thaw a vial of 10mg/ml Blasticidin.

Day 1: Titration plate set up

- 1 Detach, collect and count cells by following Steps 1-8 of the protocol: [Passaging adherent cancer cell lines](#).
- 2 Resuspend 5×10^5 cells in 5 mL of culture media, at a concentration of 1×10^5 cells/ml.
- 3 Using a 10mg/ml stock of blasticidin, prepare four dilutions, at 2x final concentration by diluting the stock in media as show in Table 1, column C & D. (When the 2x antibiotic concentration is diluted with an equal volume of cell suspension it will result in the final concentration show in Table 1, column B).

Note

- Prepare a minimum of 5ml of each 2x antibiotic to that the volume is adequate for loading a multi-channel pipette without bubbles.
- Antibiotic dilutions should be prepared fresh on the day that they are required.

2 x concentration (µg/ml)	Final concentration (µg/ml)	10mg/ml stock blasticidin (µl)	Media (ml)	Total (ml)
20	10	10	4.99	5
50	25	25	4.975	5

100	50	50	4.95	5
150	75	75	4.825	5

Table 1. Preparation of blasticidin concentrations using 10mg/ml stock to achieve a 2x concentration.

Note

A wider range of blasticidin concentrations can be used if necessary, for example 1µg/ml-200µg/ml, depending on the cell type and the sensitivity of the cell line.

Safety information

Blasticidin is toxic if swallowed and harmful in contact with skin.

- Using a multi-channel pipette, add 75 µL cell suspension to the first 3 wells of rows B-F in a 96-well plate (row A is used as a control with no cells, to subtract background luminescence).

Note

Always seed 3 wells per row as the titration is carried out in triplicate. Therefore, a 96-well plate can be used to titrate up to 4 cell lines at a time (see Fig. 1).

- Using a multi-channel pipette, add 150 µL media to the first 3 wells of row A, and 75 µL media to the first 3 wells of row B in a 96-well plate.
- Pipette 75 µL of the blasticidin 2x concentrations into the first 3 wells of rows C-F, to achieve the final concentrations as per the plate layout shown in Fig. 1.

Figure 1. Plate layout for blasticidin titration of one cell line.

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Incubate at 37°C , 5% CO_2 for approximately 72 hours.

Day 4: Assessing cell viability using CellTiter-Glo at 72 hou...

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Thaw CellTiter-Glo 2.0 reagent and equilibrate to room-temperature prior to use. Mix by gently swirling to obtain a homogeneous solution.

Note

- The CellTiter-Glo reagent can be stored at -20°C and is stable for up to 4 freeze-thaws; thawed reagent can be kept at 4°C for up to 5 months.
- CellTiter-Glo is light sensitive so should be stored in tin foil, and used in a cell culture hood with the light off where possible.

9

Remove the 96-well plate from the incubator and allow to equilibrate to room-temperature for 15 minutes.

10 Using a multi-channel pipette, add 25 μ L CellTiter-Glo reagent to each well (1:6 dilution) and mix by gently rocking the plate back and forth. Incubate at room-temperature for 10 minutes (wrap plate in blue roll/foil or keep away from light where possible).

11 Use an appropriate plate reader to record the luminescence of each well.

Note

The plate reader should be set to an integration time of 1 second per well, and optimised for a peak emission wavelength of 560nm.

12 Create a kill curve as follows:

- Average the triplicate luminescence values to get a single value for each condition.
- Subtract the average background luminescence (row A, media only) from the other averaged values.
- Divide the average luminescence for 10, 25, 50 and 75 μ g/ml by the 0 μ g/ml average to get a relative percentage viability.
- Plot these values on a graph to create a kill curve.

The 'kill concentration' is the lowest concentration of blasticidin which results in death of approximately 100% of cells after 72 hours.

For example, the 'kill concentration' in Fig. 2 is 25 μ g/ml.

Figure 2. Kill curve for a blasticidin titrated cancer cell line.