Cell Survival Rate Assay

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[Abstract] This protocol utilizes PicoGreen 96-well plate technology. This method is applied to estimate the sensitivity of different tumor cell lines to chemodrugs.

Materials and Reagents

1. 10% FBS culture medium
2. Chemodrugs
3. PicoGreen (Life Technologies, Invitrogen™, catalog number: P7581)
4. TryLE express (Trypsin) (Life Technologies, Gibco®, catalog number: 12605-010)
5. Phosphate buffered saline (PBS)
6. Deionized water

Equipment

1. 96-Well culture plate
2. TECAN Genios Instrument (PHENIX)
3. Incubator
4. Foil
5. Spectrophotometer

Procedure

1. Suck out medium, wash once with 5 ml PBS. Add 2 ml tryLE express. After cells become detached, add 10 ml complete medium. Pipette the cell suspension with 5-10 ml pipette to become single cell suspension. This step is very important (to get single cell suspension, put the pipette tip onto the bottom of the flask, then push out the cell suspension through squeezing the cell suspension out of pipette).
2. Count the cells and dilute to 1 x 10/ml, place cells in 96-well plate in 100 µl (1 x10^3/well).
3. Interested medication was diluted in a series of concentrations and was added in wells in 100 µl (the medication was prepared in 2 times of final concentration).

4. Put the plate in 37 °C, 5% CO_2 for 1 week.
5. Remove media from plate by shaking the plate in sink. Then tap on towel to remove the remaining of media.
6. Wash with PBS 200 µl/well twice. Remove PBS by shaking plate. Tap plate on towel.
7. Add 100 µl deionized water to each well. Place the plate in incubator 37 °C, 5% CO_2 for 1 h.
8. Dilute PicoGreen in deionized water 1:200 (will need 100 µl/well). Make sure to calculate how much you will need. Wrap the plate with foil and let sit for 1 h at RT (this step can be done overnight if you are busy).
9. Measure the plate with spectrophotometer for fluorescence intensity.
10. Process the data:

    Survival Rate =

    Average fluorescence intensity in experimental wells x 100%

    Average fluorescence intensity in control wells

    Note: PicoGreen *is a fluorochrome that selectively binds dsDNA and has characteristics similar to that of SYBR-Green I. It has a maximum excitation at 480 nm and emission at 520 nm.*

Acknowledgments

This protocol was adapted from Ahn *et al.* (1996) and Enger (1996).

References

1. Ahn, S. J., Costa, J. and Emanuel, J. R. (1996). PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. *Nucleic Acids Res* 24(13): 2623-2625.
2. Enger, O. (1996). Use of the fluorescent dye PicoGreen for quantification of PCR products after agarose gel electrophoresis. *Biotechniques* 21(3): 372-374.

