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SRB assay for measuring target cell killing V.1

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Protocol adapted from:
Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols **2006**;1(3):1112-6 doi 10.1038/nprot.2006.179.

Abstract

The Sulforhodamine B colorimetric assay was used to measure effector CAR T cell killing of their target tumor cells.

Troubleshooting

1 Perform co-culture experiment of activated CAR T cells and target tumor cells at various tumor:effector ratio in a 96-well plate for 24 hours at 37'C, 5% CO2.

2 Remove cell culture supernatant 24 hours after co-culture assay.

3 Using a multichannel pipet, fix adherent tumor cells on the 96-well plate with 100uL cold 10% (w/v) tricholoroacetic acid (TCA) to each well, and incubate at 4'C for 1hr.

4 Wash four times by dipping entire plate in a basin with slow-running tap water. Tap plates on paper towels to remove excess water, and allow to dry at room temperature.

5 Add 100uL of 0.057% (w/v) Sulforhodamine B (SRB) solution to each well and incubate at room temperature for 30 minutes.

6 Immediately wash plates four times in a basin containing 1% (v/v) acetic acid to remove unbound dye.

7 After the plates have dried completely, add 200uL of 10mM Tris base solution (pH 10.5) to each well and incubate for 5 minutes with shaking.

8 Read plates in a microplate reader at A510nm.

9 Calculate the percentage of cell growth inhibition using the formula below:
% cells killed = 100 - [mean OD(co-cultured sample)/mean OD(tumor cell only)]x100