Mar 28, 2025

Cell viability using PrestoBlue HS

Methods and Protocols

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4z7k2vo5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.3byl4z7k2vo5/v1

External link: https://doi.org/10.3390/mps8040075

Protocol Citation: Jacqueline Ngo-Reymond 2025. Cell viability using PrestoBlue HS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4z7k2vo5/v1

Manuscript citation:
Perier M, Wang L, Simonneau M, Ngo-Reymond J, Guillermet-Guibert J, Lafond M, Lafon C (2025) Formation and Characterization of Two Magnetic Three-Dimensional Spheroid Models of Murine Pancreatic Adenocarcinoma. Methods and Protocols 8(4). doi: 10.3390/mps8040075

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

Created: March 28, 2025

Last Modified: March 28, 2025

Protocol Integer ID: 125641

Keywords: cell viability, viability of cell, prestoblue hs this protocol, using prestoblue h, cell

Abstract

This protocol measures the viability of cells cultured in 2D.

Materials

KPC A219
iMEF
DMEM/F-12 culture medium (Gibco 21331-020) with FBS , 1%
L-Glutamine and 1% Penicillin/Streptomycin
DPBS
Trypsin EDTA 0,05%
96-well plate for culture bottom F
PrestoBlue HS Cell Viability Reagent (Invitrogen P50200)
Aluminium
Opaque 96-well plate for reading (Perkin Elmer Optiplate
96-F blake Polystyrene 6005270)
Fluorescence reader (Horiba Fluoromax-4)

1. Day one : Cell seeding

1 Cells should
not be confluent the day of test. They should be in exponentiel phase of growth.
At confluency, cells may lower their metabolic rate. So before experiment, the appropriate cell density should be determined.

2 - Warm the complete DMEM/F12 medium at 37°C

3 - Under biosafety hood, aspirate the medium in 75cm² flask

4 -Add 5 ml of DPBS to rinse the cell and aspirate the PBS

5 - Add 2 ml of trypsin EDTA 0,05% and incubate at 37°C for 5 minutes. Verify that cells were detached from the flask wall. Add 10 ml of medium and flush 3 times to separate cells

6 -Take 20 µl of cells of each cell line and count

7 -Prepare cell suspension to have 4000 cells in 100 µl for drug incubation for 24 hours and 2000 cells in 100µl for 72 hours

8 - Dispense 100 µl in each well of 96-well plate

9 - Place the 96-well plates in incubator at 37°C, 5% CO_2

2. Day 2 : Drug treatment

10 - prepare solution to have 0-50-100-200 nM of Gemcitabine

11 - Aspirate the medium in 96-well plate

12 - Dispense 100µl of differents solution

13 -Incubate for 24 to 72 hours at 37°C, 5% CO_2

3. Day 3-5 : PrestoBlue Test

14 Warm PrestoBlue HS Cell Viability Reagent to room temperature

15 Aspirate the medium in each well

16 Add 90 µl of fresh medium in each well

17 Add 10 µl per well of PrestoBlue HS Cell Viability Reagent

18 Place the 96-well plate in incubator at 37°C, 5% CO2 for 1 hour

19 Transfer medium of each well in a opaque black 96-well plate

20 Record fluorescence on plate reader (Enter value of emission (560nm) and excitation (590nm) wavelenght)