GENProtocols

Protocol

Streamlined High Throughput Drug Screening with PrimeSurface® White 384 well 3D plates

TRANSLATIONAL MEDICINE CANCER RESEARCH

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Abstract

Using PrimeSurface® white plate, cancer spheroid cells can be formed and tested for drug screening using luminescence viability assay in the same well without the need for reagent transfer. This streamlined process shortens the experimental steps and time required in drug testing thus providing a robust, reproducible and reliable assay. In this technical note, we demonstrate cell viability of 4 different tumor cells and the streamlined method of intracellular ATP measurement using CellTiter Glo® (Promega Co., Ltd).

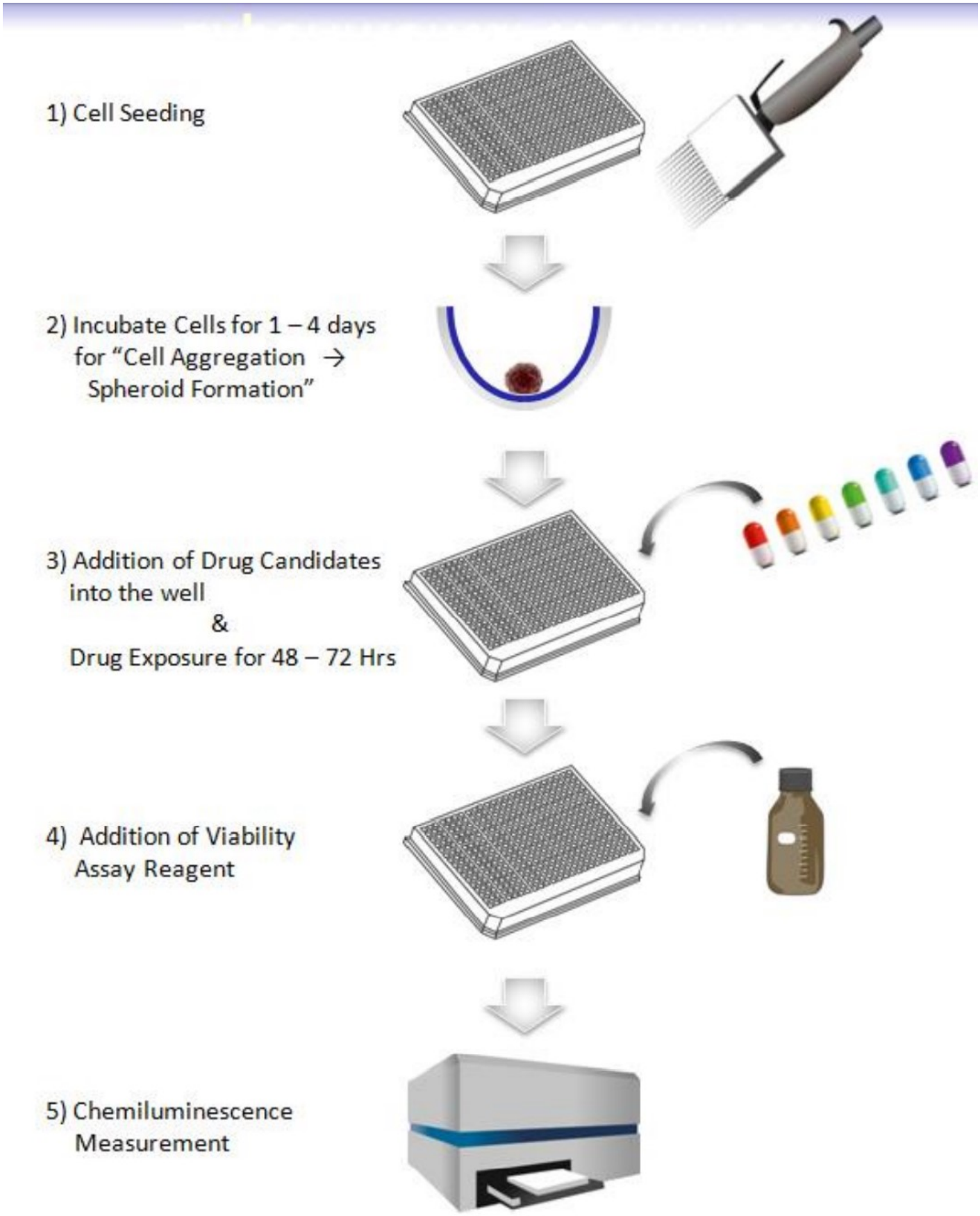
Introduction

Using PrimeSurface® white plate, cancer spheroid cells can be formed and tested for drug screening using luminescence viability assay in the same well without the need for reagent transfer.

Reagents and Equipment

1. RPMI1640 (+10%FBS +1%Penicillin-Streptomycin Mixed Solution)
2. CellTiter Glo® Luminescent Cell Viability Assay Cat. No. G7572 (Promega Co., Ltd.)
3. PrimeSurface® 384 well clear plate (MS-9384UZ, Sumitomo Bakelite Co., Ltd.)
4. PrimeSurface® 384 well white plate (MS-9384WZ, Sumitomo Bakelite Co., Ltd.)
5. Plate Reader Fusion α-FP (Perkin Elmer Co., Ltd)

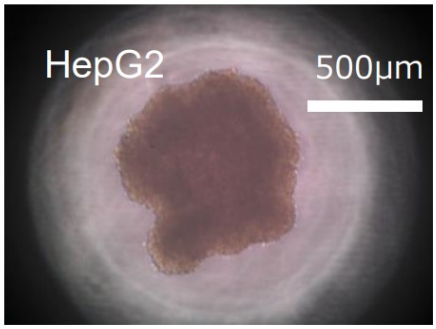
Procedure

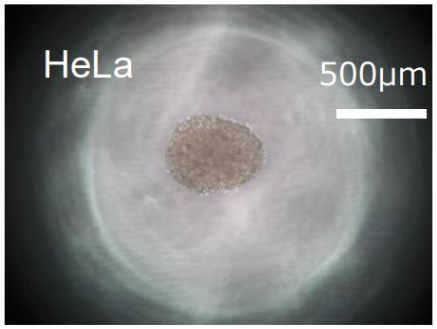


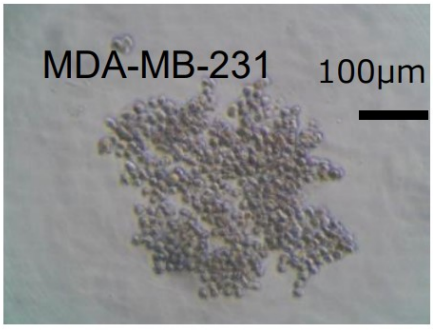
Different types of Cancer cell lines can form tight, compact or loose spheroids Four types of cells, shown below serve as a

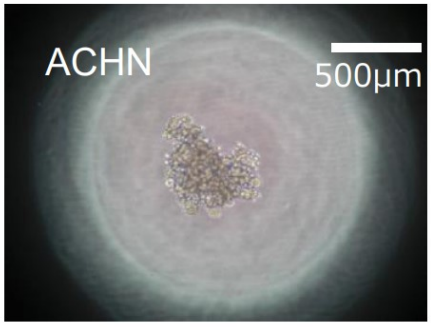
representative cases for spheroid formation. Using CellTiter Glo®, a homogeneous "add-mix-measure" reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present which is directly proportional to the number of cells present in culture.

Toughness	Cells	Origin
Tight	HepG2	Human Hepatic Cancer Cell Line
Compact	HeLa	Human Cervical Cancer Cell Line
Loose	MDA-MB-231	Human Breast Cancer Cell Line
Loose	ACHN	Human Renal Adenocarcinoma Cell Line







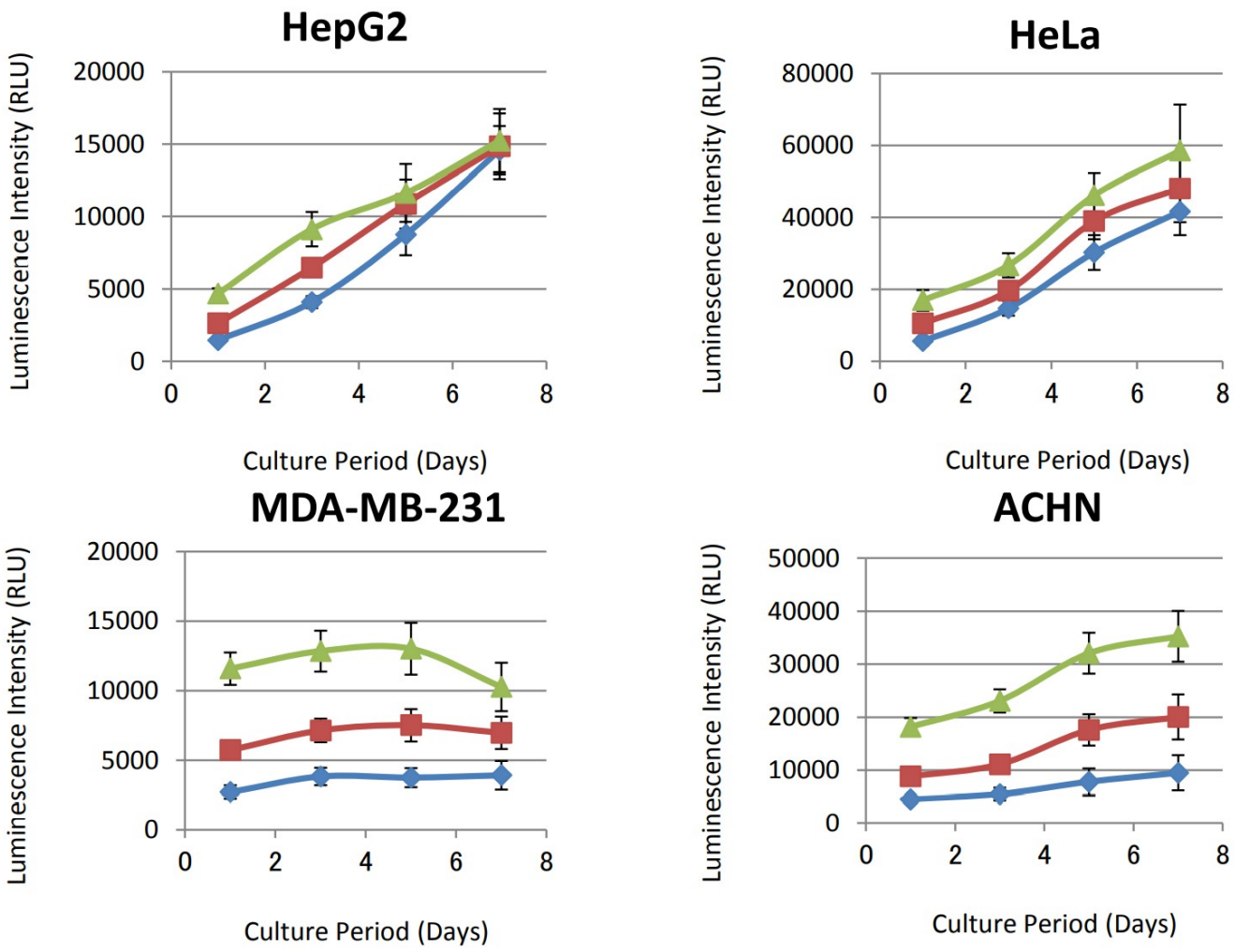


Cell Proliferation Curve

Cells were seeded in PrimeSurface® 384 well white plate (Cat. No. MS-9384WZ) with a density of 250, 500 and 1000 cells/well in 25μL of culture medium*1. Cells were incubated at 37▌/ 5□ CO2 . Luminescent intensities were measured every two days after addition of 25μL volume of CellTiter Glo® reagent and ten minutes standing at R.T.*2

*1: Based on the type of cells, number of cells, and culture medium, the amount of culture medium may need to be adjusted.

*2: If cell solubility is poor, dissolve the cells using a shaker.



Cell proliferation curve of HepG2, HeLa, MDA-MB-231 and ACHN cells (□□250cells/well, ■□500cells/well, ▲□1000cells/well)
The amount of ATP increased in the case of HepG2, HeLa, and ACHN cells with all cell seeding densities over the 7-days culture period. On the other hand, MDA-MB-231 did not show much increase in the ATP amount.

Calculation of Z'-Factor

1. Cells were seeded with 25µL/well media in PrimeSurface® 384 well white plate (MS-9384WZ) as stated above. First column and last column served as the control wells with no cells and culture medium only.
2. The intracellular ATP amount was measured at Day5. 25 µL of CellTiter Glo® reagent was added to each wells.
3. Z'-factor values were calculated as 100□ and 0□ and respectively, in wells with and without cells.

Table.1 Z'-Factor values of 4 cell types

		HepG2		HeLa		MDA-MB-231		ACHN	
plate	cells/well	All well	Inner well	All well	Inner well	All well	Inner well	All well	Inner well
PrimeSurface 384 well White plate	250	0.58	0.67	0.58	0.56	0.41	0.44	0.41	0.40
	500	0.51	0.54	0.59	0.59	0.55	0.60	0.46	0.45
	1000	0.50	0.49	0.52	0.51	0.62	0.70	0.67	0.67

Inner well : includes all inner wells and excludes outermost peripheral wells in the plate

Data above represent typical values

HepG2 and HeLa cells, having a tight spheroid formation ability, showed Z'-factors values higher than 0.5. MDA-MB-231 and ACHN cells, having a loose spheroid formation ability also showed Z'-factors values higher than 0.4

$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}.$$

σ_p : SD of positive samples, σ_n : SD of positive samples
μ_p : mean values of positive samples, μ_n : mean values of positive samples

Z-Factor values:
1 > Z > 0.5: Excellent assay, 0.5 > Z > 0: Allowable assays, Z < 0: Unallowable assays

Time Taken

Variable

References

Not available