EZ-BindShut® Application note No.1

Contents

【Key word】

SP or MPC coat: original low cell adhesion coat, 3D cell culture, tumor spheroid, cytotoxicity assay

SP coated products	: EZ-BindShut®-SP
MPC coated products	: EZ-BindShut® Ⅱ

【Introduction】

Three dimensional (3D) cell culture is more physiologically relevant than 2D culture method. 3D cell culture system has 3D cell-cell adhesion, gradient of oxygen consumption and soluble factor's penetration(1). Therefore 3D cell culture have been used in many research area such as stem cell differentiation, cancer biology, drug discovery, hepatotoxicity and organoids.

EZ-BindShut® is a 3D cell culture microplate, in which low cell adhesion polymer coated to the interior surface of the well. EZ-BindShut® has two variation of low cell adhesion polymer, SP or MPC. SP is AGC original low cell adhesion polymer, which is less soluble to water and safety tested. In EZ-BindShut® various type of cell can form spheroids in short term without forced aggregation by centrifugation. High stability of SP polymer coating will contribute reproducible spheroids generation and long-term culture of spheroids.

【General procedure】

A basic protocol of generating and culturing spheroids is described below. However, the size, growth rate and morphology are different depends on cell-type, culture medium and soluble factors.

Recommended culture volume range:
96-well:　100 to 250 µL per well

1. Prepare single dissociated cells within appropriate cell density from 2D culture of cryo-preserved vial.
2. Seed cells to the microplate respectively.
3. Incubate the microplate at 37℃ 5% CO_2.
   It is recommended that don't move the microplate for 24 hours from inoculation.
4. Change half volume of medium once or twice a week.
   Volume and frequency of medium change is depend on cell line and culture condition.

◆ Very low unspecific protein adsorption to EZ-BindShut® surface

SP- and MPC-coating demonstrate very low adsorption of bovine fibronectin and mouse IgG antibody in to the interior of well compare to the tissue culture (TC) treated surface.

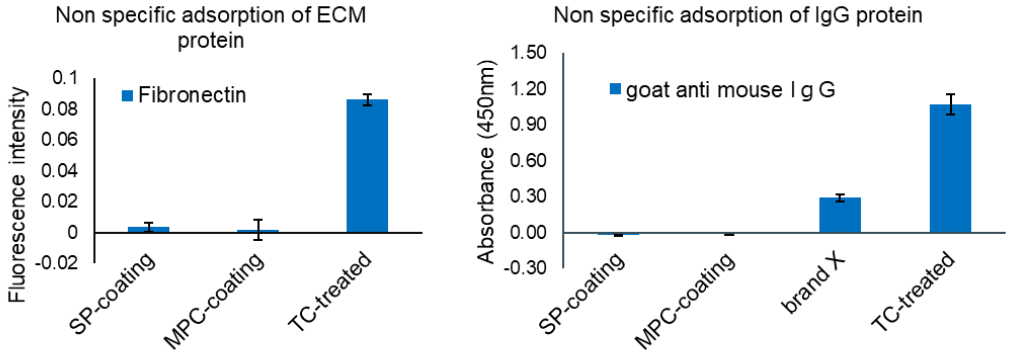

Figure 1

Wells of EZ-BindShut®-SP and –MPC and general TC-treated microplate were coated with FITC-labeled fibronectin for 22hours at 4 ℃ (n=3). Each microplates were washed with PBST (0.05% Tween20 in PBS) 3 times and then the fluorescence intensity (Ex 485 / Em 525) of the wells was measured using microplate reader Varioskan LUX (Thermo) (left graph). In the same manner microplates were coated with peroxidase labeled mouse IgG antibody for an hour at room temperature (n=4). Remaining peroxidase-IgG antibodies on the wells were allowed to be reacted with TMB color solution and the absorbance (450nm) was measured using Varioskan LUX (Thermo) (right graph). Other brand's low cell adhesion microplate is also measured.

<Product number>

EZ-BindShut®-SP 96well (U) plate : cat# 4870-800SP

EZ-BindShut® Ⅱ 96well (U) plate : cat# 4870-800LP

TC-treated 96well(U) plate : cat# 3870-096

◆ Multi-cellular tumor spheroids in EZ-BindShut® microplate

5 cancer cell lines, (and human iPS cell) spheroids were generated in EZ-BindShut®-SP/-MPC and other brand's microplates. HepG2, A549, Panc-1 and HEK293 cells formed solid spheroids and effectively grew in all microplates. However, MCF-7, breast cancer cells, only formed loose shaped cell aggregates.

Cell line	Source	Cat. No.	Tumor Type	Medium	Spheroid Morphology
HepG2	JCRB	1054	hepatocellular carcinoma	DMEM + 10%FBS	Tight
A-549	JCRB	0076	lung cancer	MEM/NEAA+10% FBS	Tight
MCF-7	JCRB	0134	breast cancer	DMEM + 10%FBS	Tight
Panc-1	ATCC	CRL-1469	Pancreas carcinoma	DMEM + 10%FBS	aggregate
HEK293	JCRB	9068	embryonal kidney cell	MEM + 10%FBS	Tight

Table 1

Overview of spheroid generation from 5 cancer cell lines.

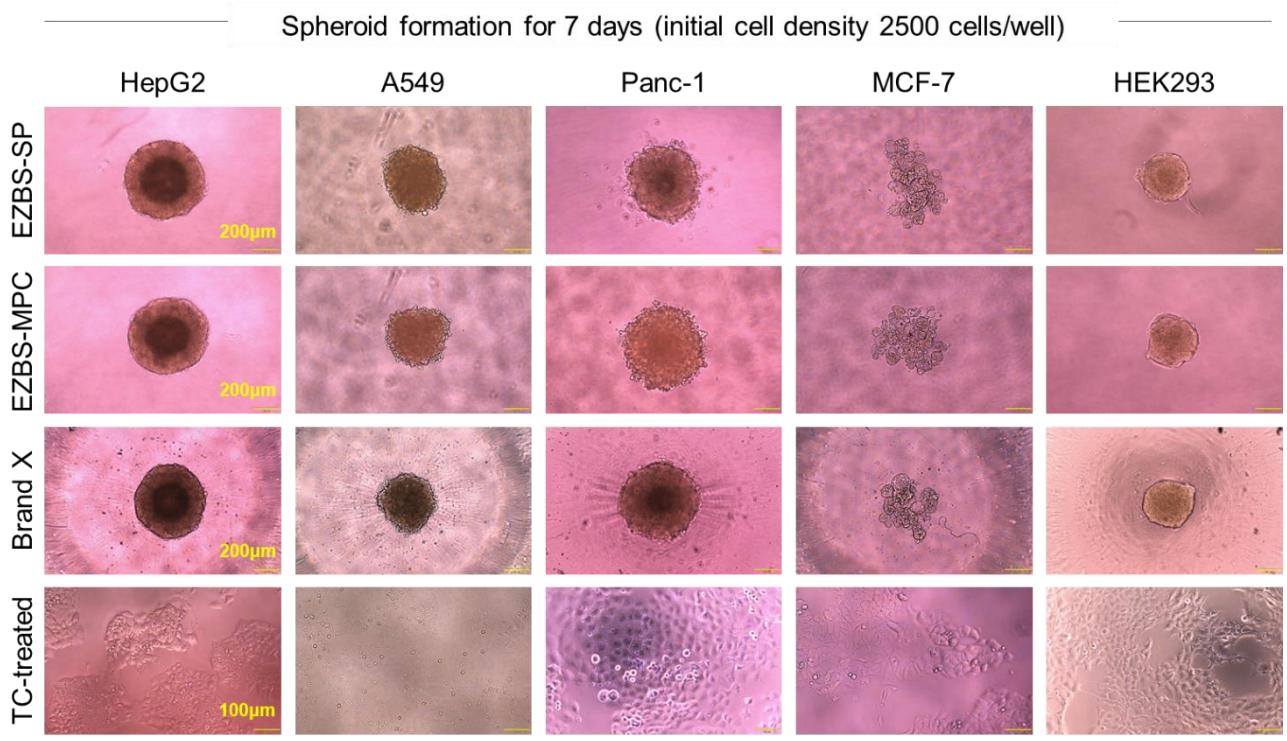

Figure 2

HepG2, A549, Panc-1, MCF-7 or HEK293 cells were inoculated at 2500 cells/well density into EZ-BindShut® (EZBS) -SP / MPC or other brand 96-well microplates and cultured for 7 days at 5% CO_2. As control TC-treated microplate was used for 2D culture. Yellow scale bars are 200μm and 100μm respectively

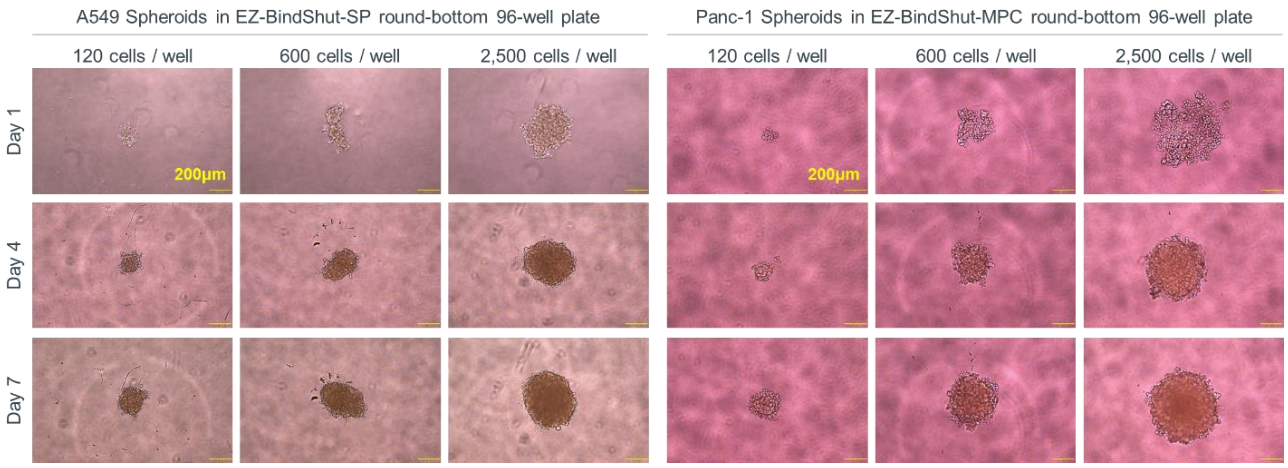

Figure 3

Example of A549 (lung cancer) and Panc-1 (pancreas carcinoma) cells spheroid formation and growth in 96-well EZ-BindShut® microplates.

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EZ-BindShut®Ⅱ 96well (U) plate : cat# 4870-800LP

TC-treated 96well(F) plate : cat# 3860-096

◆ Uniform size and morphology of spheroid generated in EZ-BindShut®

All 96 HepG2 spheroids generated in a 96-well EZ-BindShut®-SP microplate shows uniform size and morphology.

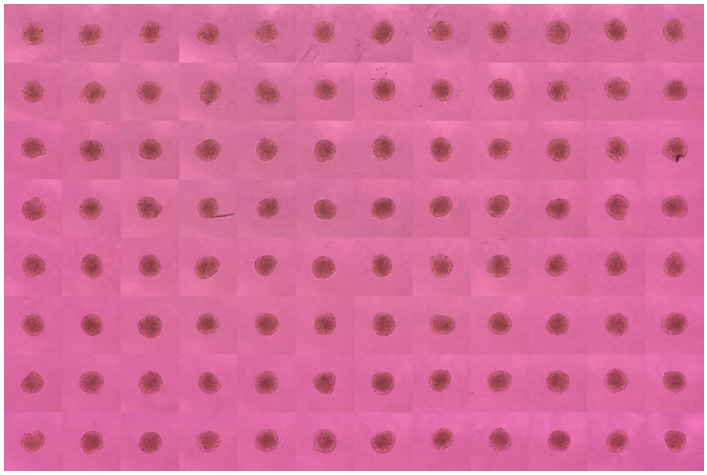

Figure4

Phase contrast images of 96 HepG2 spheroids generated in a 96-well EZ-BindShut® SP microplate.

＜Product number＞
EZ-BindShut®-SP 96well（U）plate ： cat# 4870-800SP

◆ Cytotoxicity assay in EZ-BindShut®-SP

MCF-7 dose-response to NF-κB pathway inhibitor Parteride over 3 days which was measured using CellTiter-Glo® 3D (Promega)

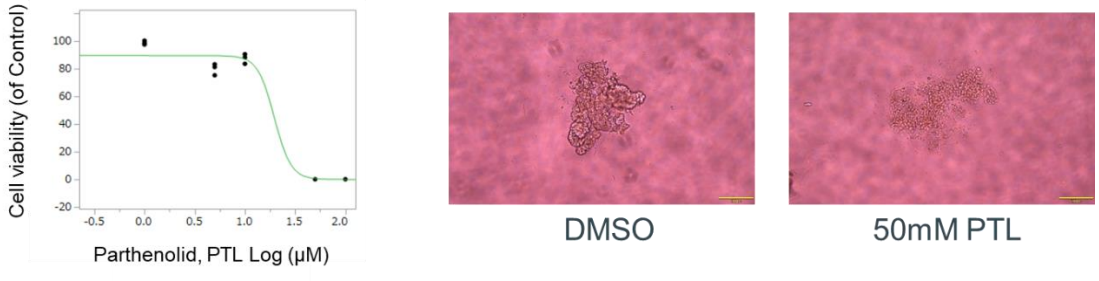

Figure4

Breast cancer cells MCF-7 aggregates were generated on 96-well EZ-BindShut®-SP microplate at 3000 cells/well density(2). From day 1 to day4, MCF-7 aggregates were treated with the anticancer drug candidate compound Parteride which is NF-κB pathway inhibitor. On day 4, to assess the viability CellTiter-Glo® 3D (Promega) was used for measuring the amount of ATP contained in living cells. As a result, a typical dose-response curve was obtained.

<Product number>
EZ-BindShut®-SP 96well (U) plate : cat# 4870-800SP

Reference

(1) Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol. 2010 Jul 1;148(1):3-15. doi: 10.1016/j.jbiotec.2010.01.012. Epub 2010 Jan 25.

(2) Zhou J, Zhang H, Gu P, Bai J, Margolick JB, Zhang Y. NF-kappaB pathway inhibitors preferentially inhibit breast cancer stem-like cells. Breast Cancer Res Treat. 2008 Oct;111(3):419-27. Epub 2007 Oct 27.