DATASHEET

Adherent Cell Kit for the Beacon® and Lightning® Systems

The Adherent Cell Kit enables the isolation, growth, monitoring and recovery of adherent cells on the Beacon and Lightning Optofluidic systems.

- Expands the use of Berkeley Lights® Platform to adherent cell types
- Selection of single cells and isolation in individual NanoPen® chambers
- Visualization of Single Cells as they grow into colonies with Time Lapse imaging
- Recovery of clonal cell populations, alive

Workflow Overview

The surface of the OptoSelect® chip is coated with an adherent cell compatible coating to enable cell adhesion and growth. The pens of the chip are coated with the adherent cell reagents while the channel of the chip is coated with a non-adherent cell coating, allowing cells and reagents to be easily manipulated by OEP™ within the channel for efficient import and export. An overview of the workflow can be seen in **Figure 1**.

Isolation and Growth of Single Cells

Cell are imported into hundreds of nanoliter-sized NanoPen chambers across an OptoSelect microfluidic chip using OEP. Single cells are identified and penned. Time lapse brightfield imaging is used to assess cell growth and clonality over the course of four days. **Figure 2** provides an example of single cell penning of HeLa cells.

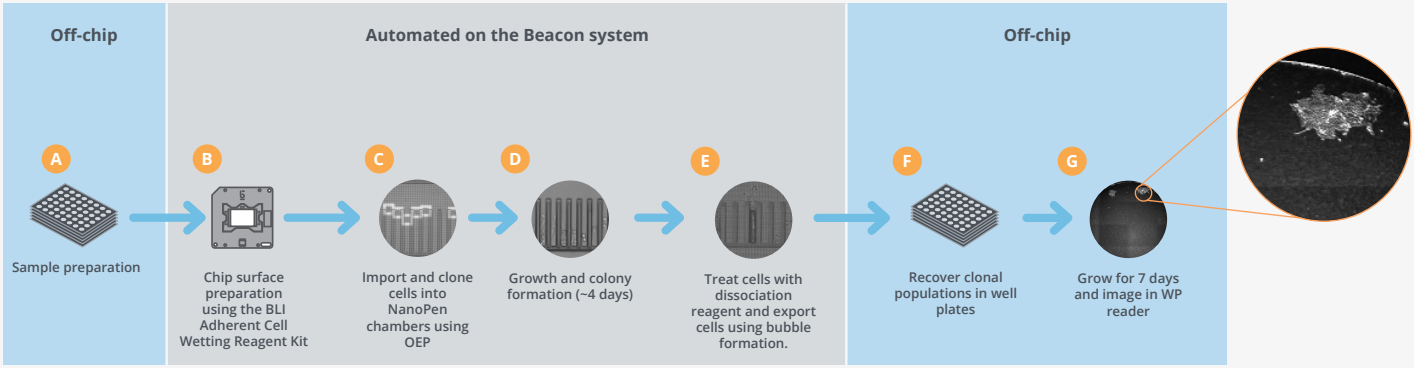

FIGURE 1: Adherent cell cloning process. Adherent Cell Workflow enables single adherent cell isolation followed by monitored growth into colonies and recovery of clonal cell populations faster than traditional limiting dilution or FACS methods.

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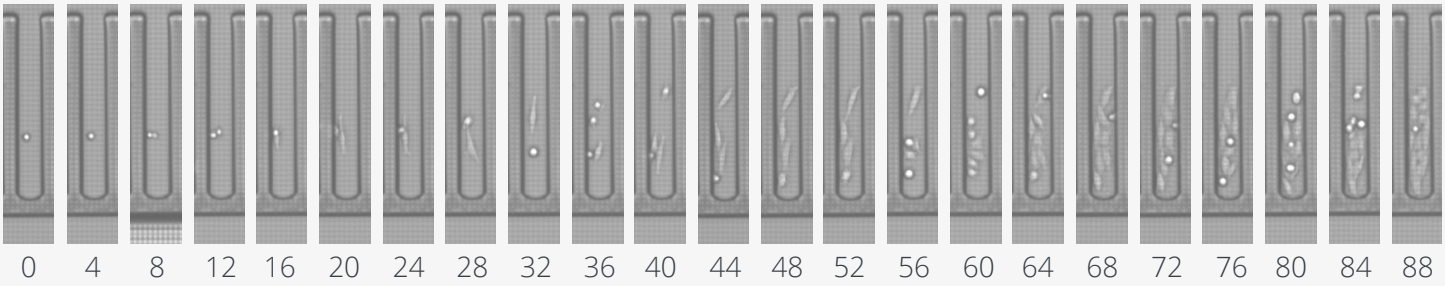

FIGURE 2: Adherent cell cloning and growth in pens. A single HeLa cell can be tracked over the full course of clonal expansion, imaged once every four hours for 88 hours.

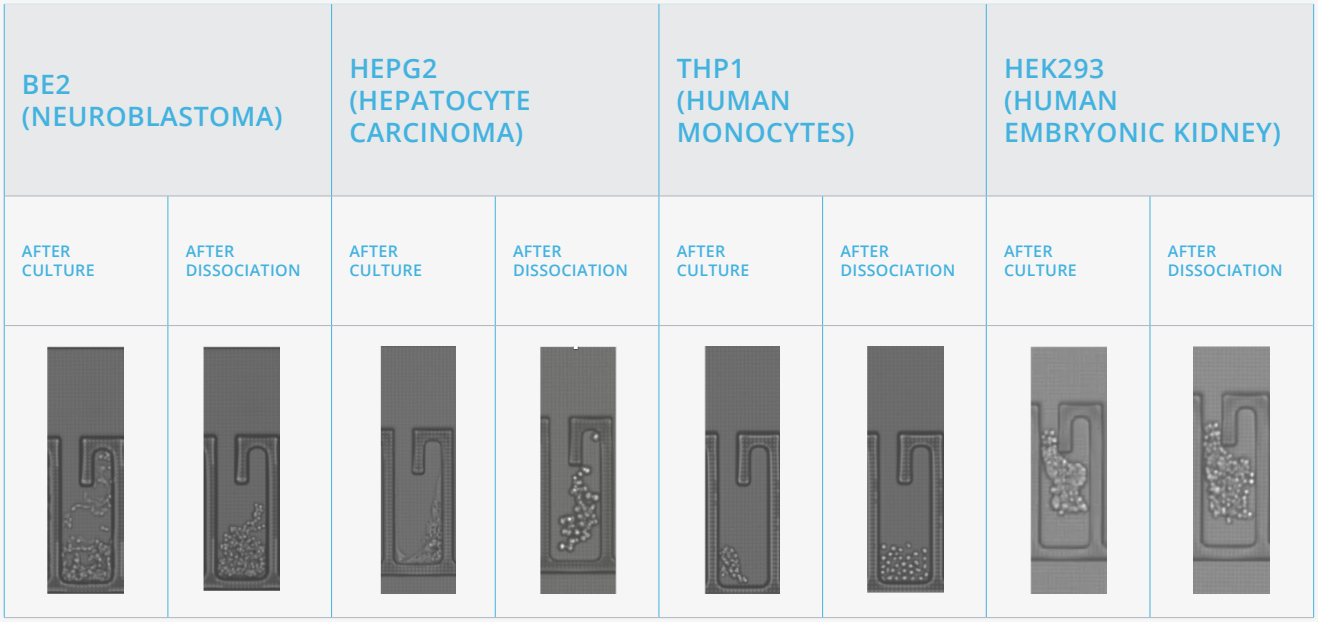

FIGURE 3: Compatible with multiple adherent cell types. The adherent cell coating has been proven to work with a variety of cell types. Images above were all taken on a Beacon system with an OptoSelect® 1750B chip. The adherent cell coating is identical for both Beacon and Lightning systems.

The coating is compatible with a variety of adherent cell types. We have successfully cloned and cultured HeLa, HEK293, HepG2 and U2OS cells using our adherent cell coating on both the Beacon and Lightning systems (**Figure 3**).

Figure 4 demonstrates how many pens receive single cells in typical penning. For purposes of exporting viable colonies, pens containing at least 8 cells are automatically identified as the best export candidates. Of the pens that are exported, we observe 97% cell outgrowth after export (**Table 1**).

FIGURE 4: On-chip viability. Averages of 8 single-chip workflows are shown. We see can successfully pen 1050 single cells per chip on average and of those single cells, 80% have doubled after 4 days.

Cell Imaging

Once cells have been grown on the OptoSelect chip, they can be stained on the system and imaged in multiple fluorescent channels to assess intracellular structure or identify organelles within the cells (**Figure 5**).

Export and Cell Recovery

Growing up single cells in well plates from limiting dilution is labor-intensive, time-consuming, and costly. The Beacon and Lightning systems enable single-cell isolation and cloning in just 1 week. Growing colonies are identified on the OptoSelect chip using automated brightfield imaging and counting.

To prepare cells for export, the chip is perfused with dissociation reagent to partially detach adherent cells from the chip surface. An air bubble is formed in the bottom corner of the pen and expanded so that the cells are gently nudged out of the pen and into the fluidic channel after which media is flushed through the channel to remove them from the chip and deposit them into a single well of a 96-well plate (**Figure 6**). The air bubble dissipates and the chip flushed a second time with media. The process is repeated for every selected pen colony.

Table 1 shows the typical export efficiency with adherent cells on the Lightning system.

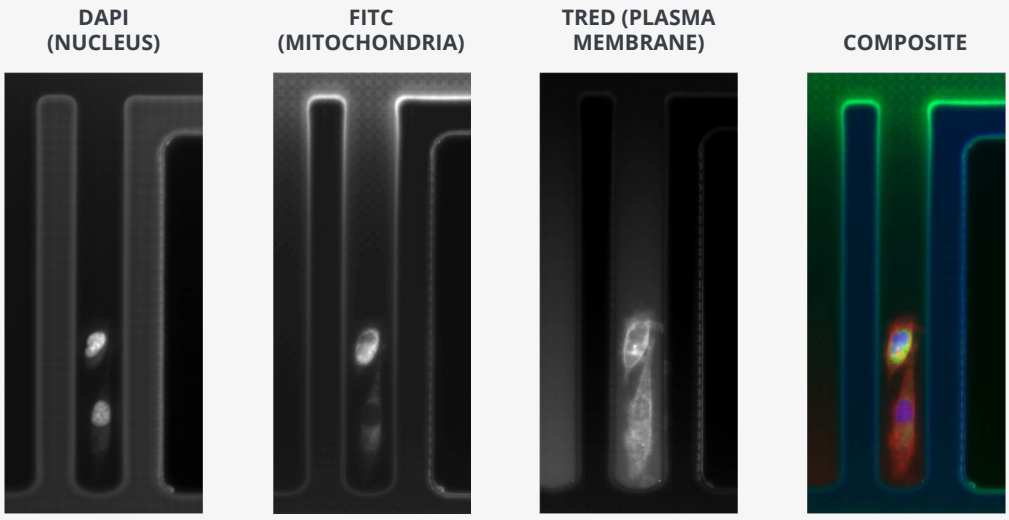

FIGURE 5: Sub-cellular staining. U2OS cells grown on the Lightning System. Cells were stained with a mitochondria stain (FITC, MitoView Green), a nuclei stain (DAPI, Hoechst) and a plasma membrane stain (Cy5, Cell Mask Deep Red) and then imaged in each fluorescent channel with the onboard 20X objective.

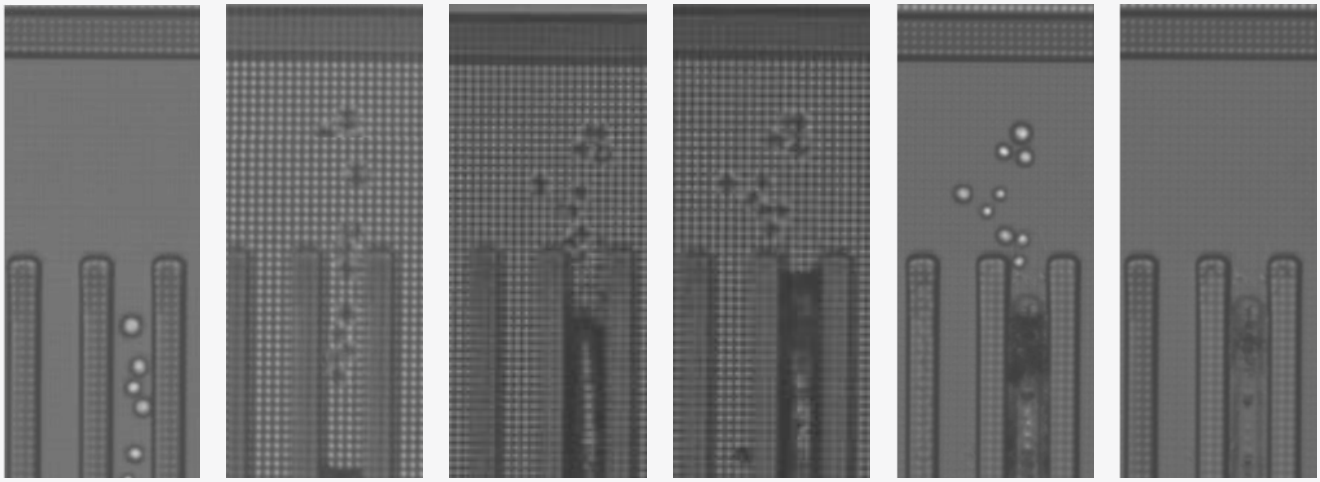

FIGURE 6: Adherent cell export. Desired clones are selected for export. Disassociation reagent is perfused through the chip to get partial detachment from the Chip Surface. A bubble is formed in the bottom of the pen, and expanded to gently move cells of a selected pen out of the chamber and into the fluidic channel. Flushing the channel with media removes the clone from the chip and deposits them into a well of a 96-well plate. After 7 days, the well plates are imaged to confirm cells have continued to grow into viable colonies in the well.

	Lightning System 1		Lightning System 2		Lightning System 3		Lightning System 4		
	Workflow 1	Workflow 2	Workflow 3	Workflow 4	Workflow 5	Workflow 6	Workflow 7	Workflow 8	Total
# of Clones Exported	24	24	24	24	24	24	24	24	192
# of Viable Clones	23	22	24	22	24	24	24	24	187
Export Viability	96%	92%	100%	92%	100%	100%	100%	100%	97%
# of Blanks	24	24	24	24	24	24	24	24	192
# of Failures	2	1	0	8	1	0	0	0	12
% Monoclonality	92%	96%	100%	67%	96%	100%	100%	100%	94%

TABLE 1: Export results. The table presents a summary of Export Results for adherent HeLa cells exported on a several Lightning systems. On average, we are capable of exporting viable cells and can confirm they grow into colonies 97% of the time.

Conclusion

The Adherent Cell Workflow Kit (750-00027) with the Beacon or Lightning Optofluidic system enables the isolation, growth, monitoring and recovery of adherent cells. The kit provides the necessary reagents to differentially coat the NanoPen chambers of the OptoSelect chip as well as the dissociation reagent used prior to clonal cell export. Using the Beacon or Lightning System to screen, grow, and recover clonal adherent cell populations is substantially faster and cheaper than traditional limiting dilution or FACS methods.

References

1 https://www.sciencedirect.com/topics/engineering/adherent-cell#:~:text=1%20Adherent%20Cell%20Culture,located%20within%20the%20connective%20tissue.

2 https://www.ptglab.com/support/cell-culture-protocol/cell-types-culture-characteristics/

3 https://www.thermofisher.com/us/en/home/life-science/cell-culture/cell-culture-plastics/cell-culture-plates.html

Ordering Information

ADHERENT CELL KIT, 750-00027

PRODUCT COMPONENT	QUANTITY
Wetting Solution	4
Adherent Wetting Solution	1
Adherent Matrix Surface (10X)	4

Kit is sufficent for 4 experiments. Kit does not include any OptoSelect chips.

Required Software

SYSTEM	SOFTWARE	VERSION
Beacon system	Cell Analysis Suite (CAS™) software	v2.1 or later
Lightning system	Cell Analysis Suite (CAS™) software	v2.3 or greater

TechAccess Subscription

Don't have a Beacon or Lightning system? We have several options for gaining access to the Berkeley Lights Platform.*

*Available in select regions.

FOR MORE INFORMATION, VISIT
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