DATASHEET
REV F | NOVEMBER 2024

Cell Line Development (CLD)

The Opto® CLD Workflow

INTRODUCTION

Cell Line Development (CLD) on the Beacon® and Beacon Select™ optofluidic system enables high throughput cloning, screening, and selection of top-performing CHO cell lines in just days. Used globally by leading pharmaceutical companies and CDMOs, the Beacon and Beacon Select systems' industry-leading technology delivers unrivaled speed and efficiency to cell line development workflows. The Opto® CLD workflow has enabled customers to select clones with higher titers than traditional methods [**Customer Spotlight: Catalent**], increase throughput by 4X while reducing their cell line development timeline by up to 50% [**Customer Spotlight: Mycenax**], and recover clones with >99% monoclonality assurance to support FDA IND regulatory filings [**Application Note: FDA Accepted IND**]. In addition, the Opto™ Assure quality assays enable users to select clones with favorable product quality attributes within 5 days of cloning to reduce overall costs, improve the probability of success, and further shorten timelines by selecting top clones for scale up.

The Opto® CLD workflow provides you:

- Selective Cell Cloning for better clones and accelerated campaign timelines
- Custom Productivity Assay for selection of cell lines expressing a wide range of therapeutic proteins and vaccines
- Opto® Assure Assays to identify clones with favorable manufacturability profiles early in development:
  - Aggregation Assay to improve process efficiency by eliminating clones that are susceptible to aggregation. See **Application Note: Aggregation Assay.**
  - Bispecific Heterodimer Assay to select clones secreting a high percentage of fully-formed heterodimer product and low levels of side products.

Beacon® System
4-chip

Beacon Select™ System
2-chip

CLONE, CULTURE, ASSAY AND SELECT TOP CLONES IN A SINGLE RUN ON A SINGLE PLATFORM

The Beacon and Beacon Select optofluidic systems from Bruker offers an integrated end-to-end automated workflow for rapid cloning and selection in mammalian cell line development. The Opto® CLD workflow allows for selection of clonal cell lines with optimal growth, productivity and quality profiles, using bioreactor-relevant on-chip assays.

During the Opto CLD workflow, transfected cells are enriched, cloned, cultured, assayed, selected and recovered in a single automated process with minimal human intervention. This is compared to conventional clone selection methods where multiple steps must be performed sequentially on many different instruments. Additionally, the Opto CLD workflow has been demonstrated to provide a cloning efficiency almost 5x greater than FACS and 10x greater than limiting dilution while offering >99% monoclonality assurance.[1]

Clone

CHO cells from one or more transfected pools are imported on OptoSelect® chips, which each contain 1,758 NanoPen® chambers ("pens") connected to a continuous fluidic path (**FIGURE 1A**). Opto-electropositioning (OEP™) visible light technology uses light to capture and gently manipulate hundreds of individual cells in parallel. The cloning operation consists of identification and sorting of single cells from the fluidic channel into the pens ("penning", **FIGURE 1B**). Up to 6,000 single CHO cells can be cloned and screened in a single 4-chip or 2-chip workflow.

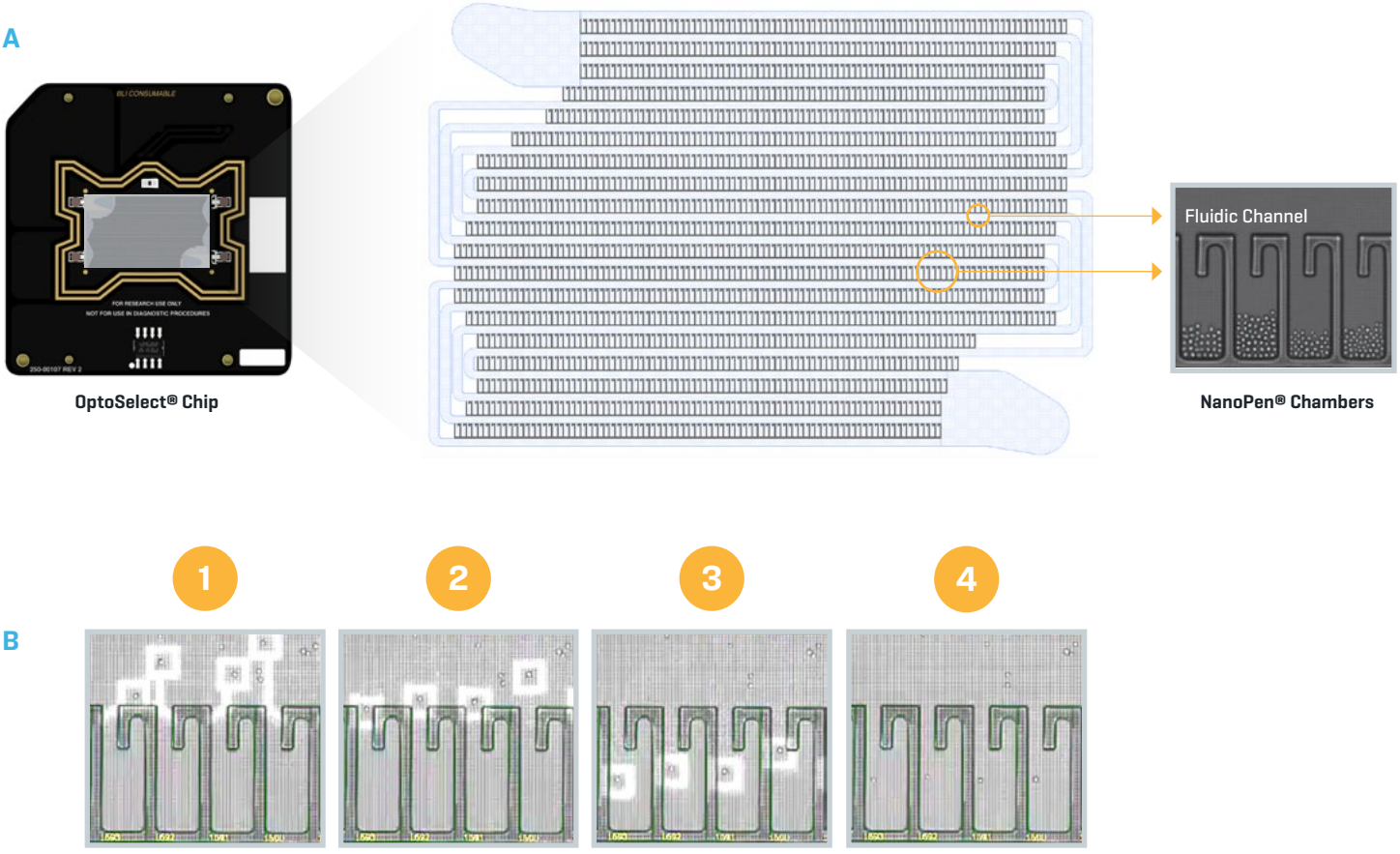


FIGURE 1: Cloning on the OptoSelect® Chip. **A]** Cells are cloned, cultured, and assayed in individual NanoPen chambers connected to a fluidic channel. Each pen is less than 2 nanoliters in volume with an imaging area over 100,000X smaller than a well in a microtiter plate. **B]** Selective penning of single cells with OEP™ visible light technology. #1, 2, 3 - Light is used to capture single cells with desired phenotypes and gently guide them into individual NanoPen® chambers. #4 - Single cells are easily visualized in pens after cloning.

Culture

A constant flow of fresh media through the channel creates an environment similar to that of a perfusion bioreactor, maintaining cell viability to enable on-chip culture over several days. Continuous automated imaging and AI algorithms monitor cell growth (FIGURE 2A). In a study published by Amgen, a cloning efficiency, which is the number of pens that had a single cell loaded and grew into colonies divided by the number of pens that expanded after recovery, 52% for the Beacon was reported.

This is almost 5 times greater than FACS and 10 times greater than Limiting Dilution (FIGURE 2B).[1] Each NanoPen® chamber is less than 2 nL in volume with an area 100,000X smaller than the well of a 96-well plate. These small dimensions reduce expansion and analysis down to the microscale so that relevant information about a clone can be obtained after just a few generations.

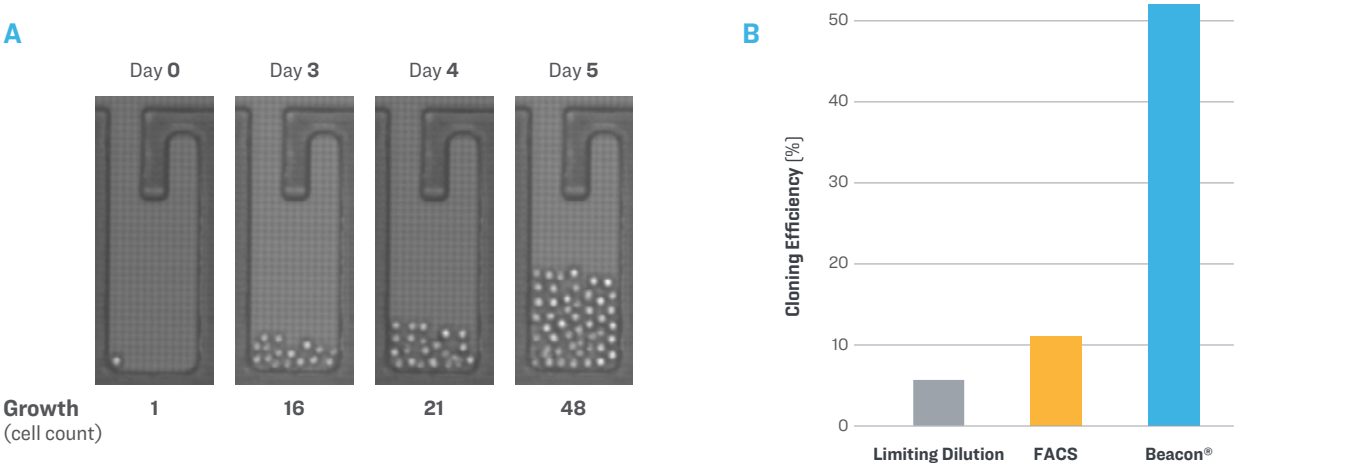


FIGURE 2: On-chip Growth and Cloning Efficiency. A) Growth is monitored over time, using automated imaging and cell counting algorithms. Cell counts and time stamps are used to calculate doubling time. B) Cloning efficiency is the number of pens that had a single cell loaded and grew into colonies divided by the number of pens that expanded after recovery. A cloning efficiency of 5.7% for Limiting Dilution has been reported, FACS was reported to be 11% and the cloning efficiency for the Beacon® is 52%.[1]

Assay

An automated sequence of fluorescence-based assays can be performed directly in NanoPen chambers over multiple time points as colonies continue to expand (FIGURE 3). Bruker's fluorescent SpotLight™ reagents bind specifically to Fc or Kappa region of human IgGs to enable on-chip quantitative productivity measurement and ranking of antibody-secreting CHO clones (FIGURE 3A). Non-destructive assays for relative titer and quality provide data on secreted product (FIGURE 3B).

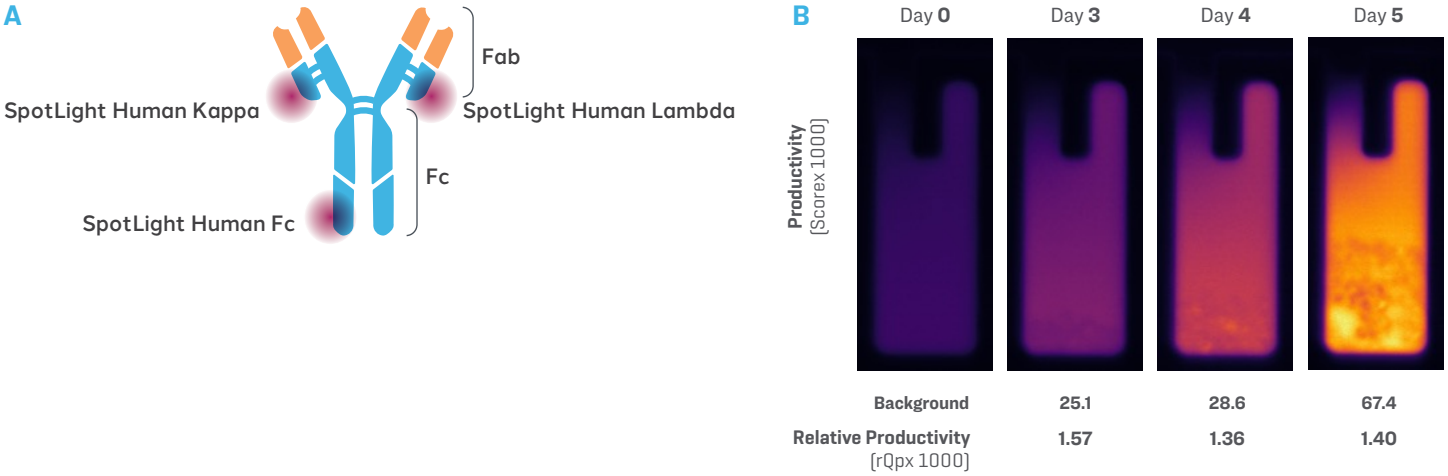


FIGURE 3: Growth and Productivity Assays. A) The SpotLight™ Human Fc reagent binds to the Fc region of human antibody molecules, whereas the SpotLight™ Human Kappa reagent binds to the kappa light chain of human antibodies. B) On-chip fluorescent assays pinpoint the highest producing clones.

C

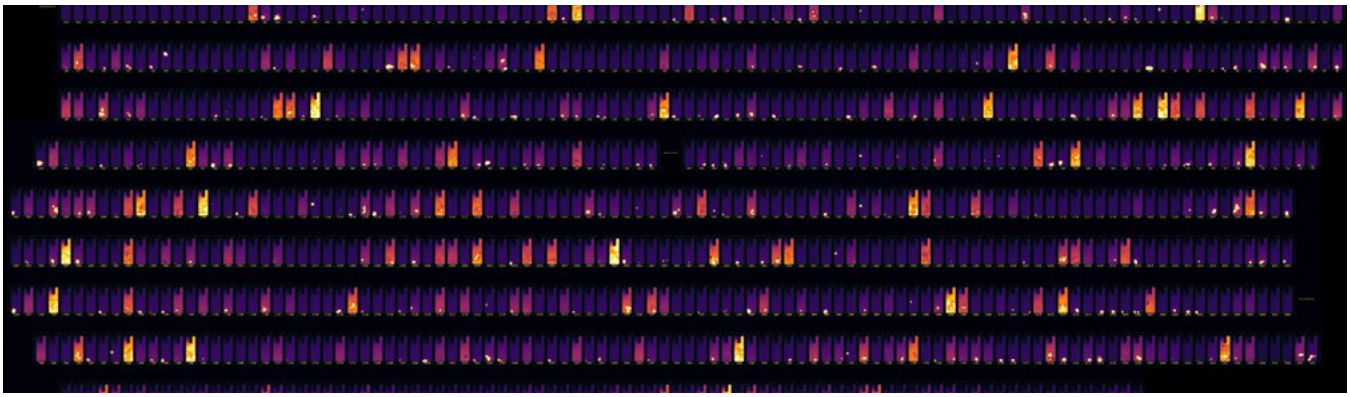


FIGURE 3C: Assays are run in parallel across thousands of NanoPen® chambers. Each chamber is assigned an assay score, which allows for ranking. A custom built software analysis tool can be used to filter the chambers. Once filters and rankings have been assigned clones can be selected based on growth and productivity scores.

Catalent demonstrated that the Opto® CLD workflow clones selected based on titer measurements on the Beacon system had 1.5–3-fold higher titers than clones selected using traditional clone picking technology when scaled up to shake flask fed-batch cultures. The titer increase was observed for two different molecules, including a traditional mAb and difficult to express protein.[2]

Select and Recover

After up to 5 days of on-chip culture and assays, desired clones are selected, recovered, and exported into microwell plates for scale up and characterization. OEP is used to direct clonal colonies out of each NanoPen chamber and into the fluidic channel. Once the cells are suspended in the channel, fresh media is flushed through to export them from the chip into a single well of a 96-well plate for scale up. This process is repeated for each of the selected clones.

Export plates are seeded with multiple cells from each NanoPen chamber, which results in clonal outgrowth as high as 100% (FIGURE 4). Automated imaging and stringent in-process controls ensure each clone is visually tracked throughout the workflow and during export so that clones are recovered with validated >99% monoclonality[1] supporting IND submission to the FDA. For more information see **Application Note: FDA-Accepted Monoclonality Assurance on the Beacon® Optofluidic System for Cell Line Development**.

FIGURE 4: Clones are Recovered for Scale Up with High Yield. Recovery of larger number of cells from each pen results in high outgrowth efficiency of exported clones. Nearly 100% of recovered clones expanded in well plates when >20 cells were unpenned per clone.

CUSTOMIZE YOUR ASSAYS FOR NON-ANTIBODY PROTEINS

With our most recent workflow enhancement, productivity assays on the Beacon or Beacon Select system are no longer limited to antibodies. The Custom Productivity Assay enables quantitative on-chip measurement of a wide range of secreted proteins such as enzymes, vaccines, fusion proteins and fragments. The Custom Productivity Assay for Opto CLD can be performed with user-provided detection molecules, ranging from small peptides to IgGs, which bind specificity to the secreted protein of interest (FIGURE 5). The Custom Productivity Assay works for a wide range of CHO-secreted proteins, including complex and difficult to express proteins. The detection molecule must be labeled with an appropriate fluorophore and quantified using standard procedures.

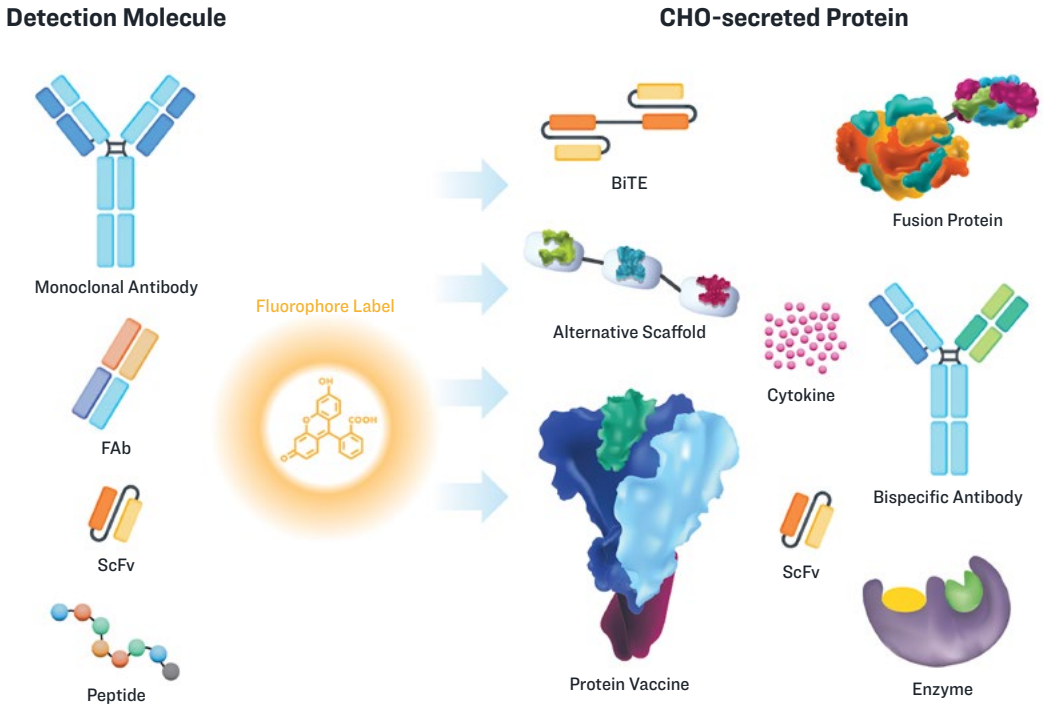


FIGURE 5: Custom Productivity Assay Enables Selection of Clones Secreting a Wide Variety of Non-antibody Molecules. The Custom Productivity Assay uses labeled detection molecules to measure a wide range of secreted proteins, including antibodies, protein-based vaccines, enzymes, fusion proteins, antibody fragments and cytokines.

Some advantages of the Custom Productivity Assay include:

- Flexibility to use your own product-specific binding molecule
- Robust for a wide range of molecules
- Enables more projects to be completed on the Beacon and Beacon Select systems

An example of success with the custom assay format is the University of Queensland who used Opto CLD to select a cell line expressing a 600 kDa molecular clamp protein COVID-19 vaccine. Within a number of weeks the team found clones with 10 times higher titer than their standard CLD workflow. The vaccine produced by a clone selected using the Custom Productivity Assay was approved for human clinical trials.[3]

INCREASE THROUGHPUT AND REDUCE TIMELINES BY SCREENING EARLIER POST TRANSFECTION

The Selective Cell Cloning (SCC) capability is a powerful enhancement to the Opto CLD workflow that integrates high-throughput analysis and sorting of cells with desired phenotypes into NanoPen chambers. SCC increases effective screening throughput by preferentially cloning cells with user-defined attributes such as viability, product secretion, morphology (*e.g.,* diameter), or surface marker expression as measured by a combination of brightfield and fluorescence imaging [FIGURE 6].

FIGURE 6: Selective Cell Cloning to Pen a Specific Population in a Heterogeneous Mixture of Cells. Two cells in the fluidic channel being targeted for cloning based on dual staining for cell viability and product expression. The left Brightfield image the cells of interest indicated by arrows. Second panel shows fluorescent staining with annexin-V, gating schemes allow cells negative for annexin-V to be preferentially selected for cloning. A separate stain indicating product secretion shows high signal for these cells, and the right panel shows them being targeted and penned with OEP.

Higher Titers Without Mini-Pools

Up to 100,000 cells can be imported, analyzed, and sorted with SCC in a single four-chip workflow. Unlike flow cytometry/FACS methods for enrichment, SCC is gentle on cells, so viability is not compromised, minimizing loss of rare clones [FIGURE 7]. This added throughput increases diversity of clones screened compared to traditional methods. Improved access to pool heterogeneity delivers higher on-chip titers and can eliminate the need for mini-pool screens - shaving several additional weeks off timeline to obtaining production-quality clonal cell lines.

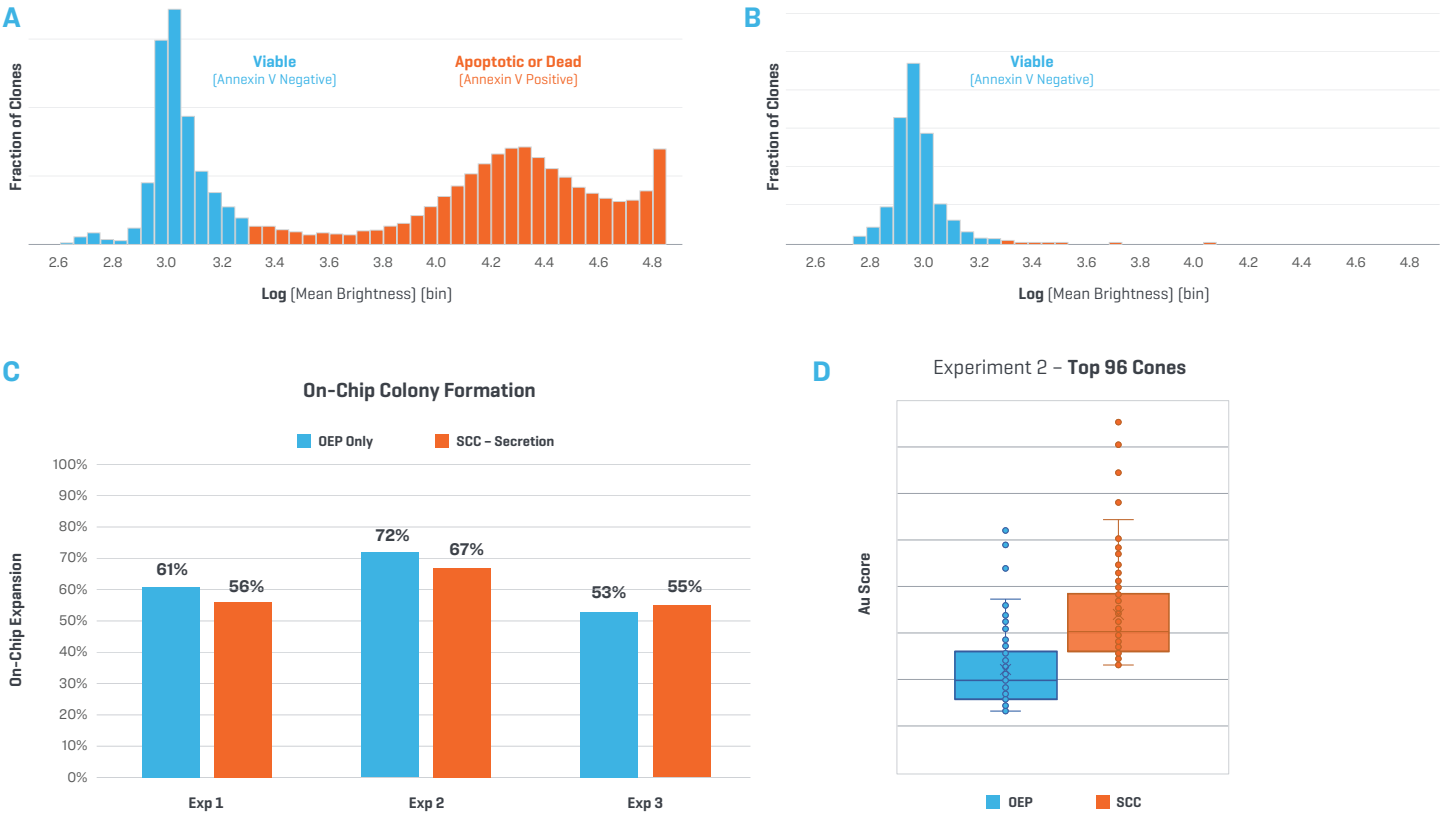


FIGURE 7: Gentle and Effective On-chip Enrichment with Selective Cell Cloning. SCC was used to enrich a mixture of viable and non-viable cells stained with an Annexin V fluorescent dye. **A)** Shows the proportion of apoptotic cells cloned with standard (non-selective) OEP penning of single cells. **B)** Shows the same pool cloned using SCC, with >97% enrichment for viable cells. **C)** Viable outgrowth of up to 70% of single cells with Opto CLD, showing minimal effect of fluorescent SCC sorting compared to standard penning. On-chip colony formation represents pens that had a single cell at loading and at least 6 cells after culture.
D) SCC enrichment leads to higher on-chip titer scores when the top 96 clones are compared to those cloned without SCC.

Clone Earlier Post-Transfection

The ability to pre-enrich for viable clones also enables the screening of pools much earlier after transfection in order to screen greater pool diversity. A recent publication by a CLD group at Amgen published in Biotechnology Progress demonstrated that Selective Cell Cloning (SCC) can be used to successfully identify and pen viable cells early in the recovery stage, when pools were at <30% viability.[4] Data on titer, VCD, specific productivity, and quality analysis at mini-bioreactor scale showed clones with comparable performance were obtained with early Opto CLD + SCC approach as with their standard method. In addition, they shortened their development timeline by 8 weeks – and achieved additional resource savings by selecting fewer clones for scale up with Opto CLD.

FIGURE 8: Cell Line Development Timeline Reduction Achievable with Opto® CLD Workflow + Selective Cell Cloning. Compared to conventional methods that require stable pool screening and selection before cloning, the accelerated Opto CLD workflow with SCC provides superior access to pool diversity by combining high throughput and minimal recovery time required post-transfection.

EARLY INSIGHTS INTO PRODUCT QUALITY: MORE INFORMATION FOR BETTER DECISIONS

Despite a growing need for earlier information on quality and manufacturability, initial clone screening in mammalian cell line development continues to focus on selection for growth and titer. Yet the fastest-growing and highest-producing clones may not secrete a product with the appropriate quality attributes. As a consequence, large numbers of clones must be expanded and characterized through repeated rounds of selection in order to maximize the probability of finding a cell line that makes high titers of manufacturable product.

The Opto Assure assay series for the Opto CLD workflow provides product quality information at the earliest stages of cell line development. Early elimination of clones susceptible to product quality issues not only increases likelihood of identifying more optimal production cell lines, but also helps speed development by reducing the number of clones that must be selected and processed through time and labor-intensive scale up characterization (FIGURE 9).

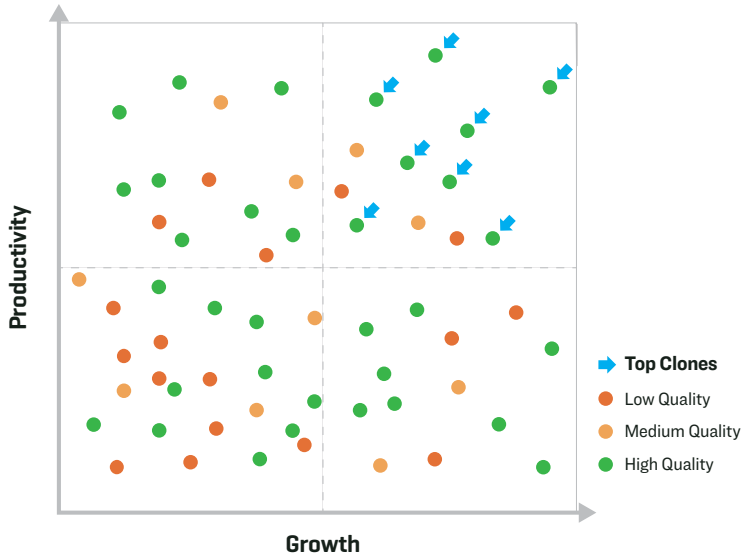
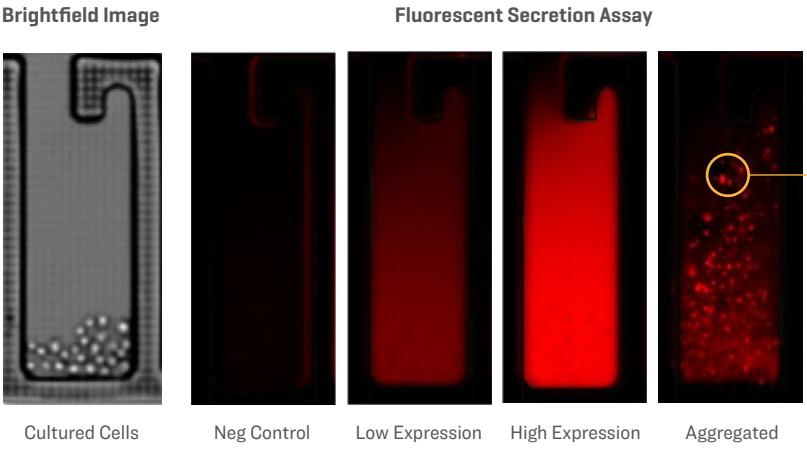


FIGURE 9: Clone Selection Based on Quality. Only top producers with the best product quality profiles are selected for initial scale up, minimizing process risk and saving valuable development time.

Aggregation Assay

Minimizing product aggregates improves process efficiency at a time when flexibility and speed to market are increasingly important. The Aggregation Assay enables automated on-chip aggregate detection to identify clones that are susceptible to product quality issues so they can be bypassed at the early screening stage.



Typically, on-chip fluorescent productivity assays will show a diffuse signal gradient in the NanoPen chambers, where the signal intensity correlates with the level of expression. Large insoluble aggregates, however, appear as fluorescent punctate spots [FIGURE 10].

Insoluable protein aggregates appear as fluorescent spots in NanoPen chambers

FIGURE 10: Early, Multi-parameter Assessment of Clones with Opto Assure Enables Early Elimination of Clones that are Susceptible to Manufacturability Issues Like Aggregation. Typically, a productive clone will show a diffuse fluorescent signal gradient in the pen where fluorescence intensity correlates to level of expression. Product aggregates, however, result in the appearance of spotted patterns.

Bruker's software detects and quantifies these aggregates, assigning an aggregation score to each clone based on count and intensity of the spots in its chamber. Clones can be filtered, sorted, and ranked based on aggregation score along with relative titer, productivity, growth, and other user-defined parameters in order to identify and export the best production cell lines.

High levels of detectable aggregates on-chip were shown to be predictive of sub-optimal clone characteristics after scale up, including lower cell densities, reduced viability, and poor product yield. For more details see **Application Note: Bring Product Quality Assessment into Early Clone Selection with the Opto® Assure Aggregation Assay**.

Bispecifics Heterodimer Assay

A significant challenge in bispecifics production is the formation of undesirable variants – improperly or incompletely assembled variants [*e.g.,* homodimers and halfmers] [FIGURE 11]. These byproducts are difficult to remove during bioprocessing and are prone to issues such as aggregation. Their presence can significantly impact product yield, quality, and safety - leading to higher downstream risk and cost.

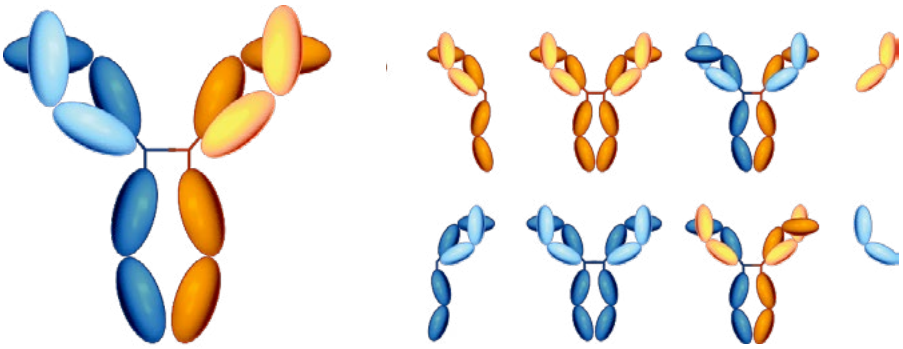


FIGURE 7: Bispecific Desired Species and Undesirable Variants. Contaminating byproducts pose challenges in downstream processing of bispecific antibodies and can significantly impact product yield of the desired species.

Each arm of a correctly assembled heterodimer binds to equal amounts of its respective target antigen [FIGURE 12A], whereas the presence of undesirable variants will bias the amount of antigen bound. Therefore, clones secreting a high percentage of heterodimer will bind equal amounts of Antigen 1 and Antigen 2. To determine the ratio of antigen binding, sequential Custom Productivity Assays use fluorescently labeled antigen molecules to measure and quantitate the binding of each antigen [FIGURE 12A]. The assays are accurately calibrated using the subunit composition of products and byproducts [*e.g.,* % heterodimer, % half-antibody/homodimer] from the pool, allowing clones with a high percentage of the desired heterodimer [*i.e.,* a ratio close to 1 and falling on the blue line in FIGURE 12B] to be quickly identified. Clones with a higher percentage of undesirable variants, for example clones producing an excess of Antigen 1 relative to Antigen 2 as indicated by the red line in FIGURE 12B have a ratio less than 1. Top producing clones that fall along the line with a slope of 1 are then selected for scale up and HPLC can be used to verify that these clones secrete a high percentage of the desired heterodimer species [FIGURE 12B].

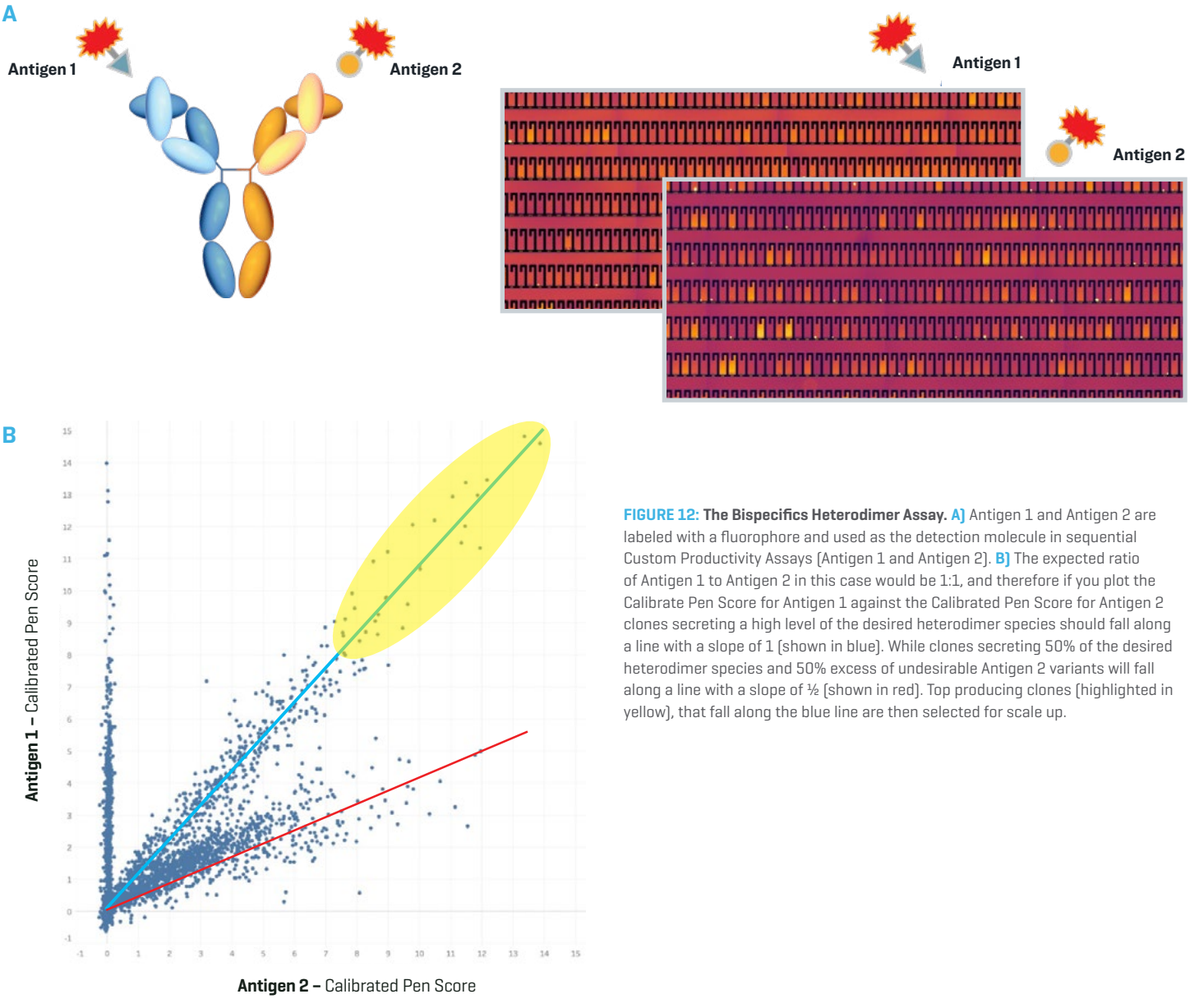


FIGURE 12: The Bispecifics Heterodimer Assay. A) Antigen 1 and Antigen 2 are labeled with a fluorophore and used as the detection molecule in sequential Custom Productivity Assays (Antigen 1 and Antigen 2). **B)** The expected ratio of Antigen 1 to Antigen 2 in this case would be 1:1, and therefore if you plot the Calibrate Pen Score for Antigen 1 against the Calibrated Pen Score for Antigen 2 clones secreting a high level of the desired heterodimer species should fall along a line with a slope of 1 (shown in blue). While clones secreting 50% of the desired heterodimer species and 50% excess of undesirable Antigen 2 variants will fall along a line with a slope of ½ (shown in red). Top producing clones (highlighted in yellow), that fall along the blue line are then selected for scale up.

For traditional cell line development workflows confidence in selected clones is low until late stages of the workflow. This requires that a large number of clones be moved through scale up [FIGURE 13A]. The Bispecifics Heterodimer Assay is a powerful tool for identifying clones that are secreting high levels of bispecific molecules within 5 days of cloning. Using this new assay, Opto CLD workflow users can identify CHO cell lines with favorable product quality profiles earlier in development, which provides high confidence in the clones that are moved forward [FIGURE 13B]. Thereby providing a reduction in overall bioprocess costs, improved probability of success, and shortened timelines by selecting fewer, higher quality clones for scale up.

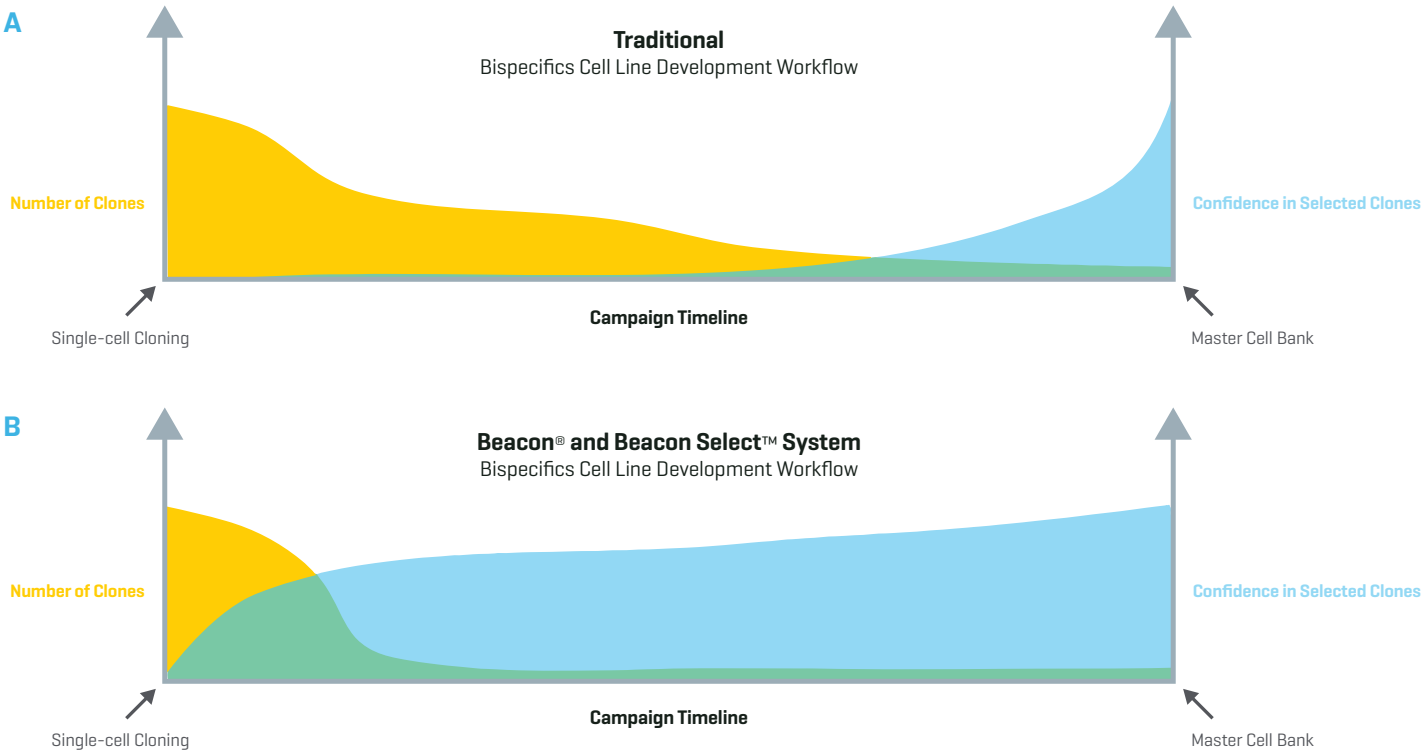


FIGURE 13: Bispecifics Heterodimer Assay on the Beacon and Beacon Select Systems. **A)** With a traditional bispecifics cell line development workflow a high number of clones must be moved forward into to scale up, due to a lack of early quality screens. Additionally, confidence in the selected clones is low until late stages in the cell line development workflow. **B)** With the Bispecifics Heterodimer Assay, which is part of our Opto Assure assay portfolio, you can identify clones with favorable product quality attributes within 5 days of cloning. This allows for an early reduction in the number of clones you move forward while providing high confidence that the clones you have selected are secreting high levels of bispecific molecules.

References

1 Le, Kim, *et al.* "Assuring clonality on the Beacon® digital cell line development platform." *Biotechnology Journal* 15.1 (2020): 1900247. https://doi.org/10.1002/biot.201900247

2 Kravitz, Rachel, *et al.* "Achieving Unique Synergies in Antibody Expression: Catalent describes the advantages of combining gene expression and clonal cell line selection platforms in cell line development." *Genetic Engineering & Biotechnology News* 39.7 (2019): 55-57. https://doi.org/10.1089/gen.39.07.16. The full article can be found here.

3 Watterson, D, *et al.* Preclinical development of a molecular clamp-stabilised subunit vaccine for severe acute respiratory syndrome coronavirus 2. *Clin Transl Immunology*. 2021 Apr 5;10(4):e1269. https://doi.org/10.1002/cti2.1269

4 Diep, J, Le, H, Le, K, *et al.* Microfluidic chip-based single-cell cloning to accelerate biologic production timelines. *Biotechnol Progress*. 2021;e3192. https://doi.org/10.1002/btpr.3192

ORDERING INFORMATION

Opto® CLD Kit, Human Fc 750-08126 – *for* BEACON SYSTEM

Part No.	Product Name	Quantity
750-00018	OptoSelect 1750b Chip*	4
520-00024	SpotLight Human Fc Reagent	
500-00030	Beacon Plastic Flush Chips	4
n/a	Wetting Additive	

** Includes Wetting Solution*

Opto® CLD Kit, Human Kappa 750-08127 – *for* BEACON SYSTEM

Part No.	Product Name	Quantity
750-00018	OptoSelect 1750b Chip*	4
520-08018	SpotLight Human Kappa Reagent	
500-00030	Beacon Plastic Flush Chips	4
n/a	Wetting Additive	

** Includes Wetting Solution*

Opto® CLD Kit, Human Fc + Kappa 750-08128 – *for* BEACON SYSTEM

Part No.	Product Name	Quantity
750-00018	OptoSelect 1750b Chip*	4
520-08018	SpotLight Human Kappa Reagent	
520-00024	SpotLight Human Fc Reagent	
500-00030	Beacon Plastic Flush Chips	4
n/a	Wetting Additive	

** Includes Wetting Solution*

Other Consumables and Reagents

Part No.	Product Name	Quantity
520-08018	SpotLight Human Kappa Reagent	
520-00024	SpotLight Human Fc Reagent	
520-08130	SpotLight Human Lambda Reagent*	
750-00018	OptoSelect 1750b Chip	
750-08298	OptoSelect 1750b-2N Chip*	
500-00030	Beacon Plastic Flush Chips	
750-08091	Wetting Additive Kit	

** Available for purchase on October 23, 2023*

Services

Part No.	Product Name	Quantity
870-02008	Service to enable Bubble Export*	

** This service is only required to access the OEP + Bubble Dislodge Unload method*

INSTRUMENTS AND COMPONENTS

Name	Description	Part Number
Beacon optofluidic system, positive pressure	6-color, standard nest lid	110-08004
Beacon Select optofluidic system, positive pressure**	6-color, standard nest lid	110-08039

*** Available access options, Capital purchase, Reagent Rental and Lease (2 year)*

SPECIFICATIONS

Import	• **Recommended input density:** 1e5 – 7e6 cells/mL • Formats: 1.5 mL Eppendorf tubes, 0.2 mL PCR tubes • Std. height (up to 16 mm) 96-well microtiter plates
Fluorescence capabilities	• Brightfield • Up to 5 colors • Standard configuration: • DAPI: Ex: 370 – 410 nm / Em: 429 – 475 nm • FITC: Ex 450 – 500 nm / Em: 515 – 565 nm • PE: Ex 540 – 557 nm / Em: 576 – 596 nm • TxRed: Ex: 542 – 582 nm / Em: 604 – 644 nm • Cy5: Ex: 608 – 648 nm / Em: 672 – 712 nm
Culture	• Customer defined media • Per chip temperature control: 10°C to 40°C

ATTRIBUTES

Dimensions	• **Width:** 46 in/116.8 cm • **Depth:** 34 in/86.4 cm • **Height:** 71.5 in/181.6 cm
Weight	• **Crated for shipment:** 1,700 lb (770 kg) • **Free-standing:** 1,260 lb (571 kg)

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