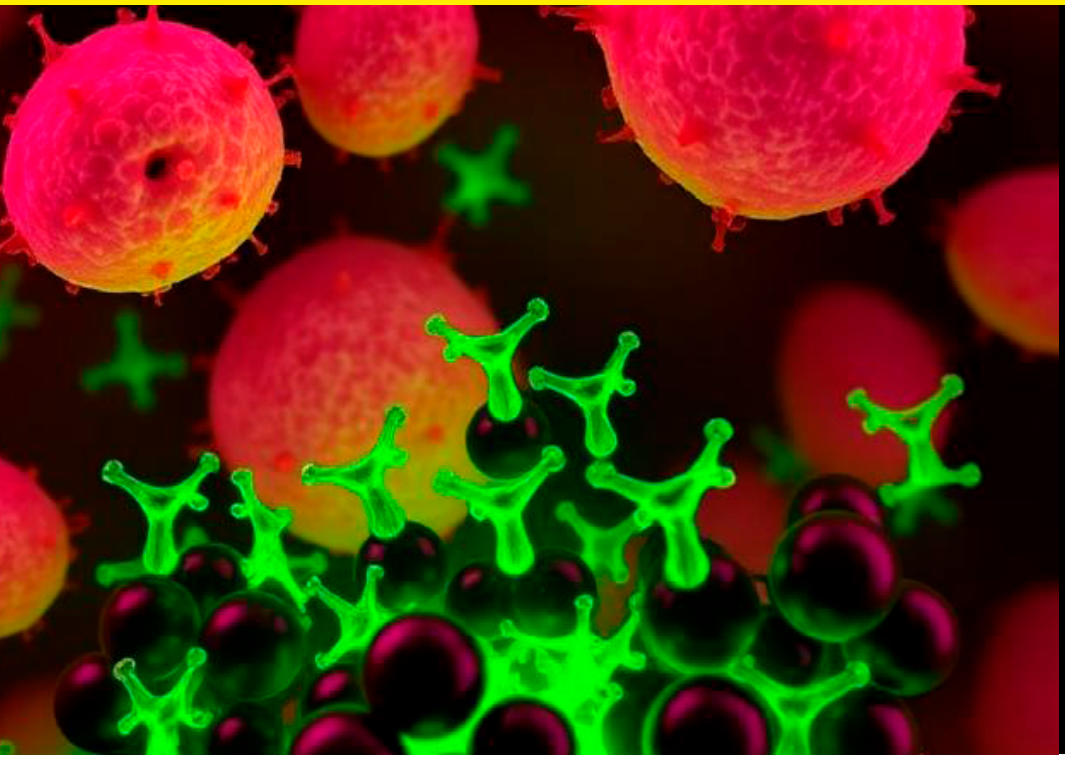
CellCelector EMA Compliant Productivity Kit for Cell Line Development

Smarter Clone Selection Starts Here



Technical Note

Scope
This technical note describes the design and utility of the CellCelector EMA Compliant Productivity Kit for Cell Line Development (CLD) for use with the CellCelector CLD in CLD workflows.

Keyword
Cell line development, monoclonal antibodies, CellCelector CLD Platform, Nanowell plate, CLD clone yield, productivity assay kit, EMA Compliant Productivity Kit for CLD, fluorescence measurements, antigen beads, secondary antibody, Incucyte® Live-Cell Analysis System

Abstract

This technical note describes the design and utility of the CellCelector EMA (European Medicines Agency) Compliant Productivity Kit for Cell Line Development (CLD) for use with the CellCelector CLD in CLD workflows. The kit allows researchers to characterize their cell clones at early stages in the CLD workflow using a novel bead-based method for evaluating productivity and growth, while documenting each element of the process. This streamlining of CLD saves resources and time, so that researchers can do more with less.

Introduction

Cell line development involves the generation of cell types with specific attributes, usually for the purposes of production of biologics such as monoclonal antibodies, or other proteins of interest. The CellCelector CLD Platform uses nanowell plate technology to streamline the CLD process incorporating an in-well productivity assay to characterize clone yield at early stages. This technical note evaluates the new productivity assay kit as a method for determining early cell clone growth and yield prior to the expensive, resource intensive expansion phase of CLD.

CellCelector EMA Compliant Productivity Kit for CLD

CellCelector nanowell plates are a proprietary technology that allow for the generation of cell lines in a much smaller footprint than conventional CLD methods. Each macrowell of a nanowell plate contains thousands of nanowells large enough to contain single cells up to small colonies. Due to the layout of these plates, cells can share media and signaling molecules, so although the cells are separated, they are not isolated. This benefit facilitates the application of the bead-based productivity assay by allowing the addition of the beads and secondary antibody globally to all individual nanowell clones within the same macrowell.

The EMA Compliant Productivity Kit for CLD from Sartorius characterizes cell clones early in the CLD workflow, by quantifying their productivity through fluorescence measurements. Briefly, the antigen beads and the secondary antibody are added to the nanowells containing the producer cells, the secreted antibody from the producer cells binds to the bead and the fluorescent secondary antibody binds to the secreted antibody (Figure 1). High yield clones will present with a higher fluorescent signal than their low yield counterparts, allowing the researcher to select the most productive for expansion.

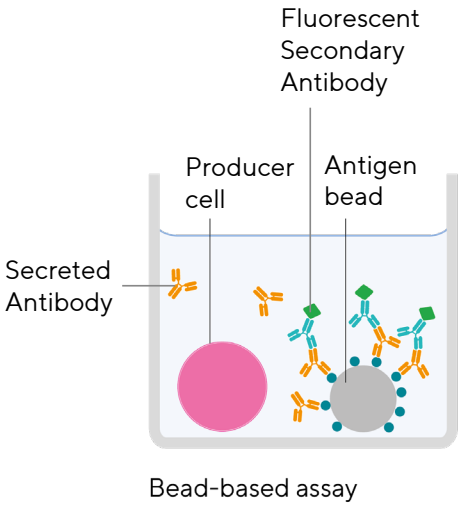


Figure 1. CellCelector EMA Compliant Productivity Kit for CLD functional mechanism. *Producer cells secrete antibody which binds to the antigen bead, the fluorescent secondary antibody binds to the secreted antibody creating a bead-antibody complex, which results in a bound fluorescent signal.*

Required materials

Material	Supplier	Catalogue number
Next Gen ULA nanowell plate	Sartorius	CC0094
Package SingleCell 80 glass capillaries	Sartorius	CC0008
EMA Compliant Productivity Kit for CLD	Sartorius	CC1317

Experimental Workflow

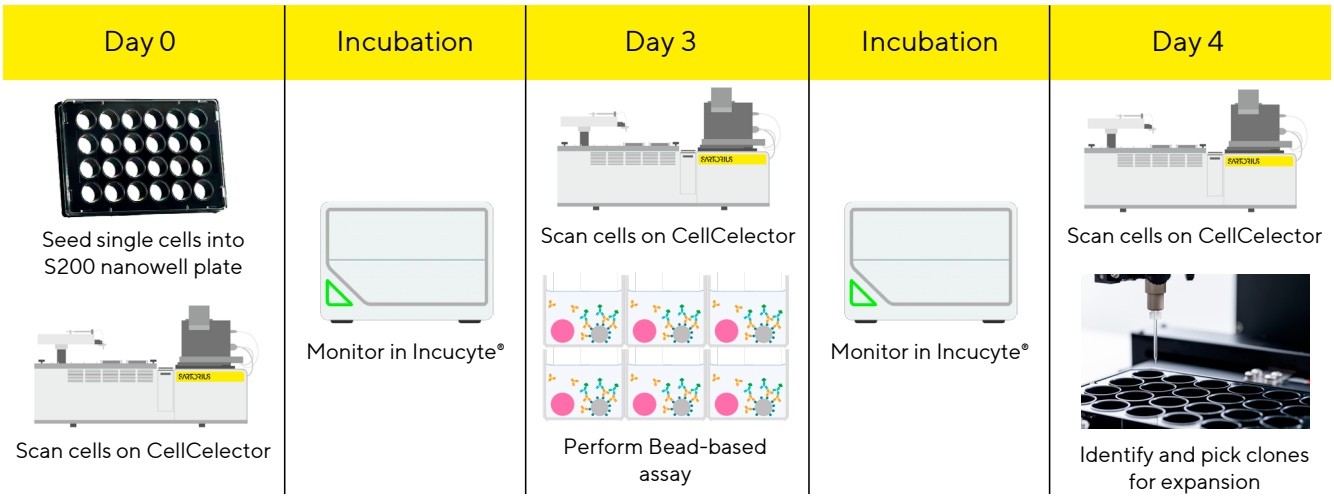


Figure 2. Bead-based productivity assay workflow.

Case Study Experimental Design

As per the workflow (Figure 2), DG44 CHO cells modified to secrete XOLAIR® (Omalizumab) were cultured in 4Cell® XtraCHO Production Medium and seeded in a single cell suspension in Next Gen S200 24-well nanowell plates at a density of 2000 cells per macro well on Day 0. Following seeding, the plate was incubated at 37°C and 7.5% CO_2 for one hour prior to scanning on the CellCelector CLD. Post scanning, the plate was incubated inside an Incucyte® Live-Cell Analysis System at 37°C and 7.5% CO_2 for monitoring until Day 3. On Day 3, the bead-based productivity assay was performed after an initial scan of the plate. Briefly, the Antigen beads were mixed with the Secondary antibody green in culture medium at concentrations of 100 µg/mL and 10 µg/mL, respectively. The antibody/bead cocktail was then added gently to each well of the assay plate being used. Once again, the plate was returned to the incubator/ Incucyte® system until Day 4. The following day, the plate was scanned on the CellCelector CLD, and clones were ranked based upon their growth from Day 0 to Day 3, and the level of fluorescent signal detected on Day 4. Once ranked, the clones of choice were picked and transferred to larger format plates for expansion.

Results

Images taken during CellCelector CLD scans (Figure 3) show the progression of clonal growth from a single cell on Day 0 to small clones on Day 3. Day 4 images show the addition of the productivity kit, and the associated fluorescent signal used to determine productivity of each clone. Finally, the before and after picking images highlight the complete picking capability of the CellCelector CLD, and the documentation of the whole process, from seeding to picking for each clone.

During the workflow, metrics such as clone growth from Day 0 to Day 3 are analyzed, while the fluorescent signal on Day 4 is calculated and cross referenced with growth metrics to determine the predicted best clones for expansion. This analysis allows the researcher to eliminate low yield clones early in the workflow, providing a pool of higher producing clones for downstream expansion and processing.

An optional step during the incubation steps of the workflow is to monitor the clones in an Incucyte® system (Figure 2). This allows clones to be visually monitored during the workflow to determine the optimal time to perform the productivity assay and to monitor growth, phenotype, and clone health.

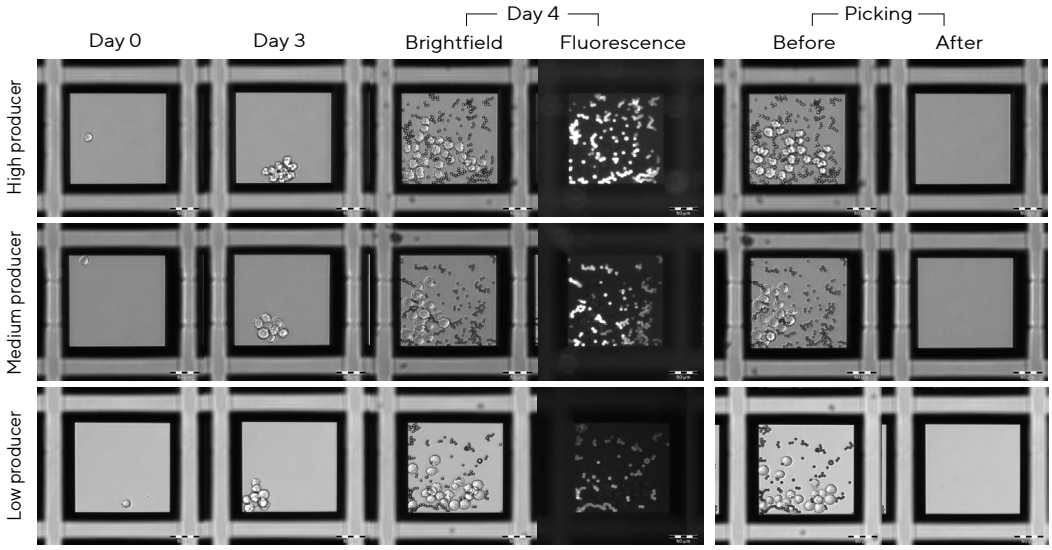


Figure 3. Scanning images taken during the EMA Compliant Productivity Kit workflow. *Images from each timepoint during the productivity workflow, including picking documentation and fluorescence imaging (black and white camera). Three examples shown, with 'High producer', 'Medium producer', and 'Low producer' fluorescent signal evaluation. Scale bar – 50 µm.*

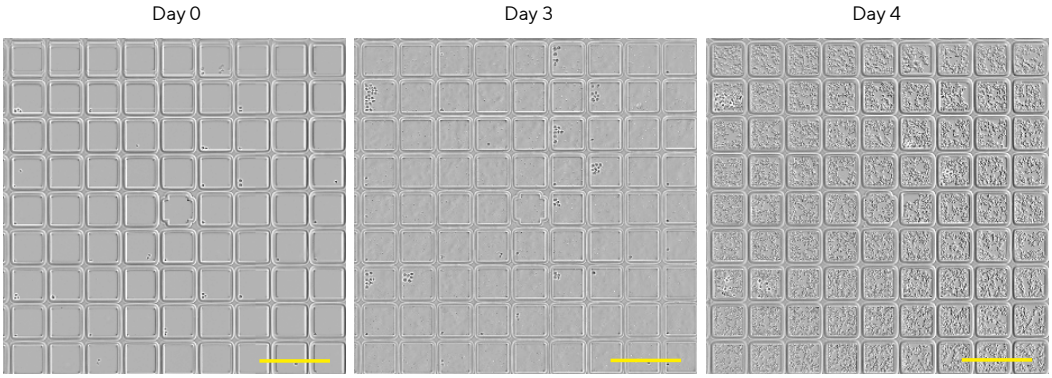


Figure 4. Incucyte® system monitoring of clones during productivity assay. *Nanowell plates were placed into the Incucyte® system during the incubation steps in the workflow. Images taken at Day 0 - post seeding, Day 3 - post addition of the bead-based productivity assay, and Day 4 - prior to analysis and picking. Note the settling of the beads from Day 3 to Day 4 into the nanowells. Scale bar – 400 µm.*

Summary

The CellCelector EMA Compliant Productivity Kit for CLD, in conjunction with the CellCelector CLD, provides early-stage prediction of better performing clones from a pool of cells. With confirmed, documented evidence of monoclonality, there is assurance that the clones produced are derived from a single cell. While the ability to rank clones based on key metrics such as growth and productivity provides researchers with a novel solution to the challenges associated with CLD workflows. Reduction in resource requirements and the time needed to reach early-stage selection milestones, means that viable clones can be generated in less time and at reduced cost.

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