SARTORIUS

Protocol

August, 2026

Description:
Optimal clone growth and productivity assessment using the EMA compliant productivity assay for CLD.

Keywords:
Reagent preparation, standard or Next Generation Nanowell plates, seed cells, CellCelector, secondary antibody, antigen beads, clone growth, monoclonal verification, Nanowell post picking

EMA Compliant Productivity Kit

For Cell Line Development (CLD)

Background

The EMA Compliant Productivity Kit for Cell Line Development (CLD) facilitates the early characterization of clone productivity within the CLD workflow using fluorescent measurements to identify and select high producing clones for further expansion. Furthermore, this kit complies with EMA/410/01 guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary products.

Introduction

This kit is specifically designed for use with proprietary CellCelector Nanowell plates. Each macrowell of a Nanowell plate comprises of thousands of Nanowells large enough to contain small colonies. Due to the unique layout of these plates, cells share media and signalling molecules whilst being kept physically separate to ensure strict monoclonality, thus promoting enhanced growth rates compared to standard microplates.

This feature therefore allows the antigen capture beads and the secondary antibody contained within the EMA Compliant Productivity Kit for CLD to be added to all individual Nanowell clones within the same macrowell. The secreted antibody from the producing clone binds to the capture bead, with the fluorescent secondary antibody binding to the secreted antibody (Figure A). Highly producing clones will therefore present with a higher fluorescent signal than their low yield counterparts, allowing researchers to select the most productive clones for expansion.

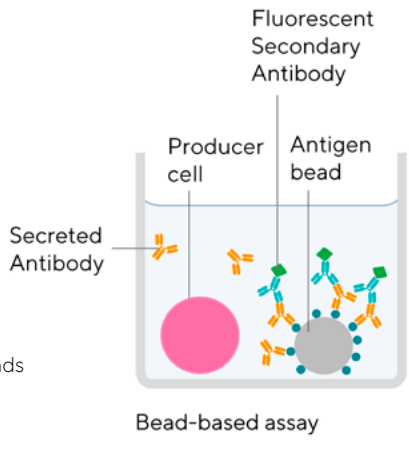

Figure A. Bead-based productivity assay functional mechanism.
Producer cells secrete antibody which binds to the antigen bead, the fluorescent secondary antibody binds to the secreted antibody creating a bead-antibody complex, which results in a bound fluorescent signal.

Kit Components

Kit Component	Component Size	Component Features
Antigen Beads	2 mL	4.0 – 4.9 µm bead size
Secondary Antibody Green	0.50 mg	Rabbit anti-Human IgG

Reagent preparation

Rehydrate 'Secondary antibody green' with 400 µL dH_2O (centrifuge if not clear) to make a final antibody concentration of 1.25 mg/mL. Prepare the working dilution on the day of use. Stable for 6 weeks at 2-8 °C after rehydration. Alternatively, aliquot and freeze at -70 °C or below for 1 year. Avoid repeated freezing and thawing.

Protocol

Quick Guide

Day 0

1. Prime either Standard or Next Generation Nanowell plates by adding 500 µL of culture medium and centrifuging the plate for 5 minutes at 1000 x g, then rotate the plate 180 degrees and centrifuge again for 5 minutes at 1000 x g to remove any bubbles collected in the nanowells.
2. Seed cells in a single cell suspension in either Standard or Next Generation Nanowell plates at 1000 cells per well in 1 mL of medium. Cell suspension should be added to each macro well in a Z shaped pattern, then centrifuge the plates for 5 minutes at 300 x g. Incubate the plates for 30 minutes to allow cells to settle.
3. Scan plates on the CellCelector after centrifugation to characterise and record monoclonality (Figures 1a and 2a).

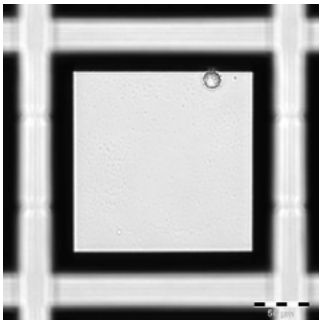

Figure 1a

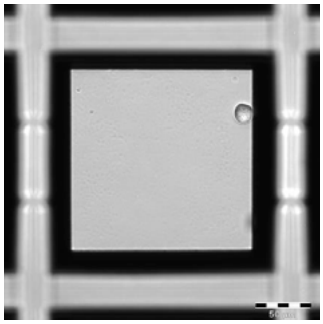

Figure 2a

Fig. 1a and 2a: *Examples of image-verified monoclonal cells.*

4. After scanning, place plates in the incubator at the settings suitable for your cell type for 3 days.

Day 3

5. On Day 3, scan plates to analyse growth (total area) prior to performing the bead-based assay (Figures 1b and 2b).

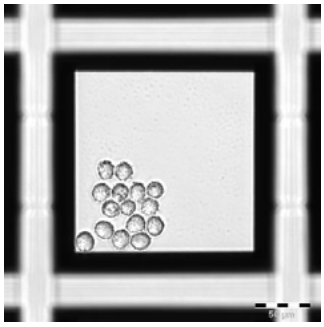

Figure 1b

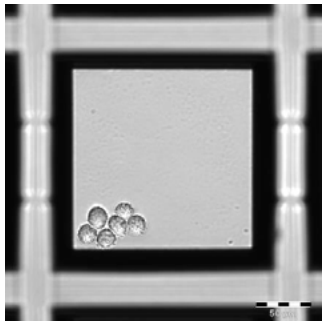

Figure 2b

Fig. 1b: *Example of a fast growing clone;* **Fig. 2b:** *Example of a slow growing clone.*

6. After the Day 3 scan, start the bead-based assay to determine which clones produce the highest yields of antibody for selection. The next steps provide details of this process.
7. Prepare the detection mix by adding 12 µL of 'Secondary antibody green' and 30 µL of 'Antigen beads' per 0.5 mL of culture medium. 0.5 mL is added per well, so multiply the reagent volumes by the number of wells you are assaying.
8. Add 0.5 mL of the detection mix in small droplets by distributing them gently over the well. Be careful not to create liquid jets or touch the surface of the medium. Do not centrifuge the plate but let the beads sediment by gravity.
9. After the beads and antibody have been added to the wells, place the plates back into the incubator overnight.

Day 4

10. On Day 4, scan plates to identify those single cell derived clones with the highest levels of fluorescence (Day 4 - average fluorescence intensity; Figures 1c and 2c) and growth rate (Day 3 - total area), based on data from all of the scans.

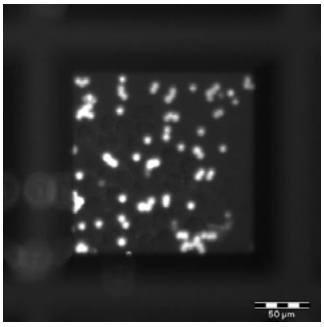

Figure 1c

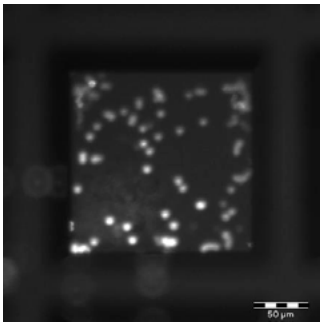

Figure 2c

Fig. 1c: *Example of optimal clone productivity (green fluroescence);*
Fig. 2c: *Example of poor clone productivity (green fluorescence)*

11. Once the selections have been made, the clones with the right characteristics (high growth rate and high average fluorescence intensity) can be picked and seeded into 384 or 96-well ULA plates for expansion. Examples of Nanowells following picking can be found in images 1d and 2d.

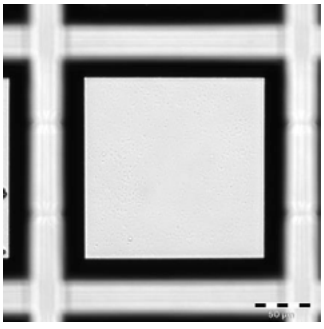

Figure 1d

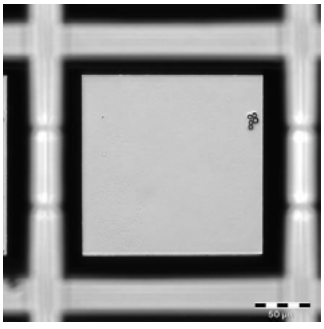

Figure 2d

Fig. 1d and 2d: *Examples of Nanowells post picking*

12. Place expansion plates into the incubator and monitor over time to determine the best growing clones.

Troubleshooting and Glossary

Step 7.
The volumes of the antibody and antigen beads have been optimised for a specific CHO cell line, please titrate the beads and antibody if these initial volumes do not work for you.

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