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Application Note

Enriching High Titer Antibody-producing Clones using Cold Capture and the Namocell Single Cell Dispenser

The Challenges of Cell Line Development for Antibody Production

Cloning of Chinese hamster ovary (CHO) cells expressing a recombinant antibody of interest is a crucial step in the development of monoclonal antibody (mAb) therapeutics. However, the heterogeneity of CHO cells can lead to a range of mAb production among individual cells in culture. To maximize efficient mAb production, it is important to identify clones that produce high IgG titers. The process of finding high IgG-producing clones typically involves screening large numbers of clones, which can make it laborious and time-consuming (FIGURE 1).

After transfecting CHO cells with the DNA to express the product of interest, the cells reach a stable pool in which some cells express the product of interest. Then, cells are selected, typically with methotrexate amplification of destabilized dihydrofolate reductase selection marker.[1] Selected cells are then isolated into single cells to generate clonal populations. These clones need to be screened and further selected for expansion. A larger fed batch is then created and tested for a variety of qualities before cells are banked for long-term storage and future use.

Single cell cloning and clone selection are among the most crucial steps in cell line development and the most challenging. These challenges include:

1. Cloning efficiency, with limited evidence of clonality
2. CHO cell heterogeneity can give rise to varied IgG expression levels among individual cells
3. Finding high-titer clones typically involves screening large numbers of clones, making it a laborious and time-consuming process

Without adequate analytical tools, the efficiency of finding the right clones can be low. The limiting dilution method may be used to dilute a population of cells to one cell per well, but limiting dilution results in a Poisson distribution of cells per well resulting in poor efficiency. As a highly manual technique, the reliability of limiting dilution is low and it's very time-consuming. FACS is a classical technique that requires a large amount of starting material and the high sorting pressure can induce significant levels of cell stress. Due to high complexity and high maintenance, FACS instruments are typically housed in core facilities and operated by specially trained staff, making it inconvenient for most biology scientists to access on a day-to-day basis.

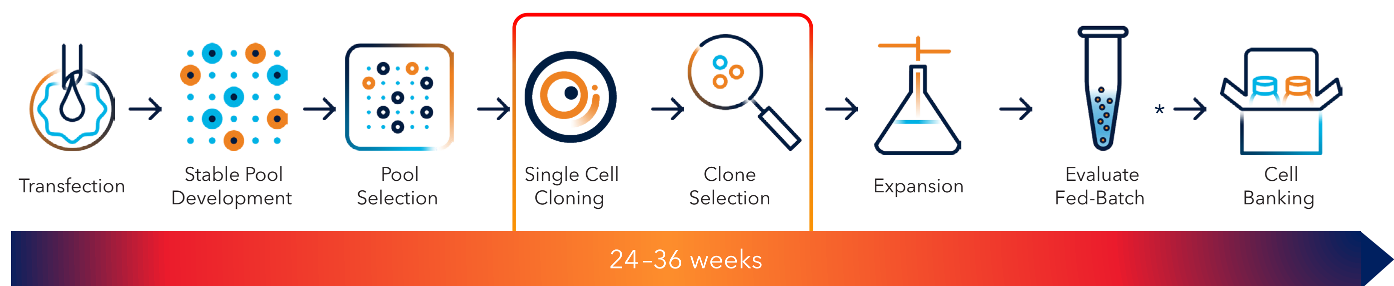


FIGURE 1. The cell line development workflow. Single cell cloning and clone selection are among the most crucial steps in cell line development but are among the most challenging.

Namocell Redefines Single Cell Isolation

Namocell is a next-generation cell sorter that solves the common challenges associated with limiting dilution and FACS methods for single cell isolation (FIGURE 2).

While FACS can sort many cells in bulk, Namocell is ideal for fast sorting of single cells into plates with a limited starting material of as little as 100 cells. The Namocell Single Cell Dispensers are easy to use, requiring no specialized training and no routine maintenance (TABLE 1).

The Namocell Single Cell Dispenser is a benchtop single cell isolation tool for any laboratory.

	Namocell	FACS
Sorting pressure	< 2 psi	20 - 70 psi
Set-up time	< 5 minutes	45+ minutes
Ease of use	Easy	Complex
Cell input	Minimal 100 cells	100,000+ cells
Droplet size	1 µL	Nanoliter
Sterile sorting	Easy	Difficult
Cost	$	$$$$
Ideal for	• Fast sorting of single cells into plates • Single cell cloning • Sterile and contamination-free sorting • Limited starting material • Rare cell isolation <0.1%	Sorting large number of cells in bulk

TABLE 1. Comparison of Namocell versus FACS for cell isolation.

The Namocell Advantages

- Namocell uses disposable, sterile cell cartridges to prevent contamination (FIGURE 3).
- Its integrated microfluidics design on the cartridge eliminates the need for lengthy calibration and uses near-zero dead volume.
- It supports sorting in culture media, keeping cells in a most optimal condition during sorting.
- The closed system prevents aerosol formation, requiring no special safety hood

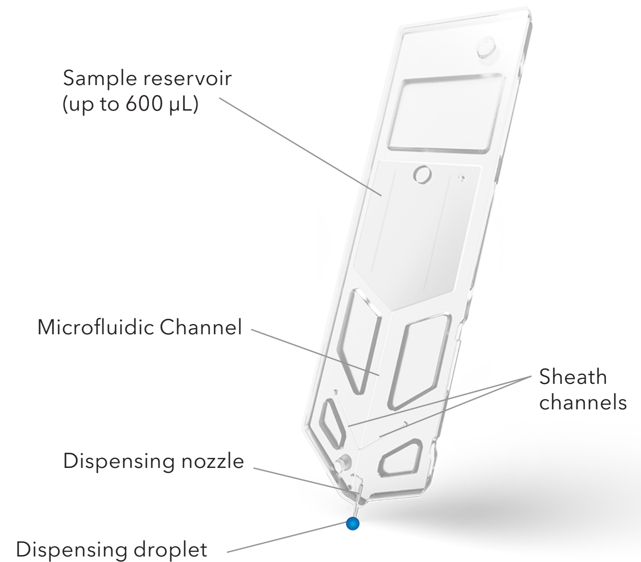
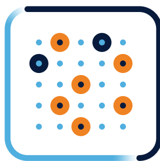


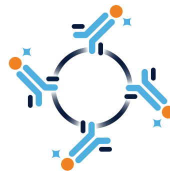
FIGURE 3. The Namocell microfluidic cell cartridge. The cell cartridge is pressurized with less than 2 psi, causing cells to travel down the center microfluidic channel with sheath entering from both sides. Individual cells passing the laser spot are gated and measured via detection channels. Target cells are sorted at an intersection, and then dispensed into a plate with preloaded culture media for single-cell cloning.

Limiting Dilution



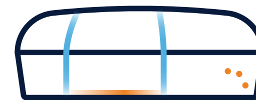
- Poison distribution
- Poor efficiency
- Poor reliability
- Time-consuming

Flow Cytometry



- Large starting material
- Sorter-induced cell stress
- Difficult to operate
- Time-consuming
- High maintenance

Namocell



- 100 cells minimum input
- Gentle sorting, healthier cells
- Easy to use
- Fast –1 min for 96 wells
  No maintenance

FIGURE 2. Methods for single cell isolation. Traditional methods (limiting dilution) versus Namocell.

The Cold Capture Method for Identifying High Titer Clones for mAb Production

In CHO cells producing antibodies, antibodies are produced in the ER, moved through the Golgi apparatus, into the secretory pathway, and ultimately released into the cell supernatant. The cold capture method was developed as a cell surface labeling approach to label secreted proteins with fluorescent antibodies at a low temperature.[2] As cells are cooled, more protein becomes stuck at the surface of the antibody-producing cells, which can be leveraged to enable labeling of antibodies while they are present on the cell surface. This facilitates identification of high-producing clones by enriching for better-producing cells for cloning, hence alleviating some of the downstream screening challenges.

During cold capture, IgG-producing CHO cells are first cooled down using cold media exchange. This causes protein secretion to slow and more proteins are trapped at the cell surface (FIGURE 4). Next, a fluorophore-conjugated capture molecule is added. This capture molecule is specific for the secreted protein of interest, and in the example here, it is an anti-IgG antibody. Finally, cells with bound capture molecules are isolated.[2]

Several studies have shown that the cold capture method improves clone survival and had broad applicability to over 2 dozen cell lines and protein products.[2-6] However, these methods relied on FACS for single-cell isolation and sorting, which has many limitations, as described above.

What if the Cold Capture Technique Could be Improved by Replacing FACS With Namocell?

In this Application Note, we develop a cold capture method using the Namocell Pala™ Single Cell Dispenser instead of FACS to improve the cloning of high titer CHO cells (FIGURE 5). We show that Namocell sorting of cold-captured cells labeled with the Human IgG PE-conjugated Antibody resulted in efficient enrichment of high-producing IgG cloneswith 3-fold increase in IgG production compared with clones without cold capture (unlabeled). Overall, the combination of cold capture and single cell sorting using Namocell is a simple and cost-effective way to improve efficiency in identifying and isolating clones for high-yield IgG production in CHO cells.

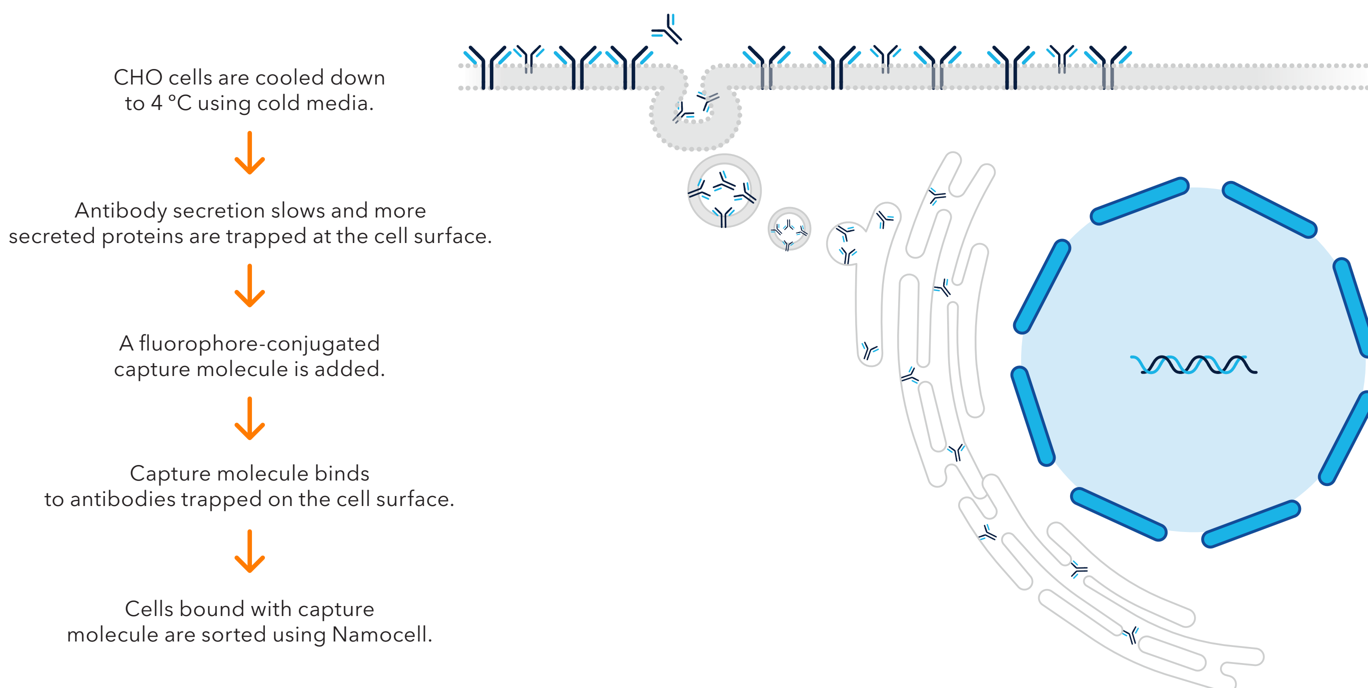


FIGURE 4. The cold capture technique for labeling anitibody-producing CHO cells in CHO cells. The cold capture method was developed as a cell surface labeling approach to label secreted proteins with fluorescent agent at a low temperature. As cells are cooled, more protein becomes stuck at the protein-producing surface of the cells, allowing the secreted proteins to be labeled with a fluorescent agent on the cell surface.

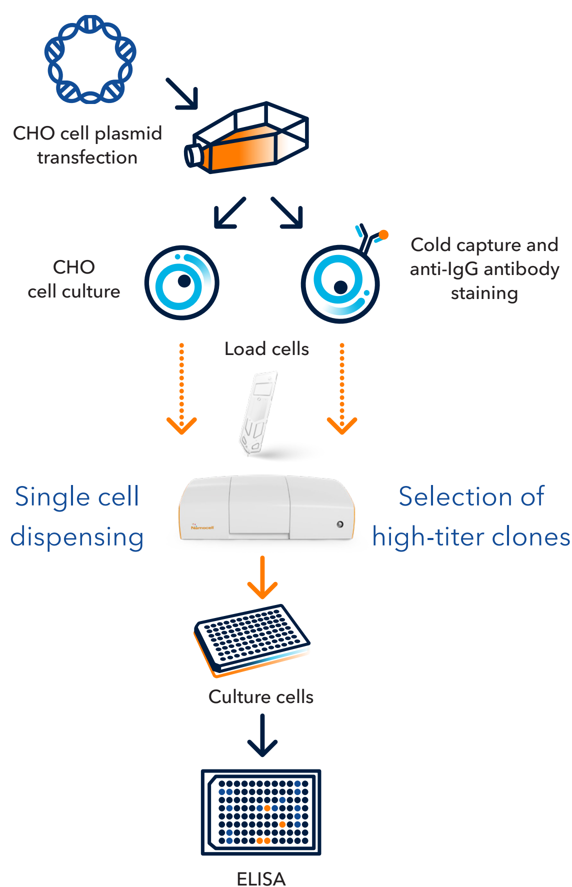


FIGURE 5. **Bio-Techne offers most of the reagents and tools necessary to complete cold capture single selection.** Transfected CHO cell pool stably expressing the monoclonal antibody of interest are isolated with or without cold capture and PE-conjugated anti-IgG antibody staining (Bio-Techne, F0157). Following selection with Namocell (Bio-Techne, NI006) and clonal expansion, colonies are analyzed via ELISA (Bio-Techne, NBP3-14790) to determine antibody titers.

Materials and Methods

The materials used in this study are listed in TABLE 2. Cells with top 5% PE fluorescence signal are selected and dispensed as single cells with the Namocell Pala Single Cell Dispenser. Sterilization is part of the recommended protocol and doesn't need to be called out here.

Cold Capture

96-well plates were prepared by adding 200 µl of sterile water to the outer wells of the plate and 200 µl of warm cloning media to the rest of the wells. A total of 12 plates were prepared in this manner. When CHO-S stable pool cells (EL186) reached a density of ~10^6 cells/mL, the supernatant was extracted and stored at 4°C to measure stable pool IgG titer by ELISA. Then, 2 mL of cells (~10^6 cells/mL) were removed and centrifuged at 100 x *g* for 3 minutes at 5 °C. The supernatant was discarded and 2 mL of chilled CDM4CHO culture media was added, and the cells were gently resuspended, and this was repeated twice. The resuspended cells were kept on ice. Then, 1 mL of resuspended cells were transferred into a pre-chilled tube, and 50 µL of PE-conjugated anti-human IgG antibody was added, thoroughly mixed, and incubated for 20 minutes on ice in the dark. Then, the cells were centrifuged at 100 x *g* for 3 minutes at 5 °C. The supernatant was discarded and 2 mL of fresh ice-cold CDM4CHO culture media was added, and the cells were gently resuspended.

The labeled cells were counted and diluted to 10,000 cells/mL with ice-cold CDM4CHO culture medium and kept on ice. The remaining unlabeled cells were counted and diluted to 10,000 cells/mL with ice-cold CHM4CHO medium and kept on ice. The Pala Quick User Guide was followed to dispense the top 5% labeled cells (draw gates: X axis-FSC, Y-axis-PE/DsRe) and unlabeled cells (draw gates: X axis-FSC, Y-axis-TSC) into the 12 previously prepared plates, 6 plates for labeled cells and 6 plates for unlabeled cells.

The clones were cultured without shaking for 3 weeks at 37 °C and 5% CO_2. After 3 weeks, cells were examined under a microscope for cell growth. The evaporation of the outer well was monitored weekly, and water was added as necessary. The wells with high and low density of cells were identified, and the supernatant from these cells was collected and stored at 4 °C for IgG titer determination.

Item	Vendor	P/N
Namocell Pala	Bio-Techne	NI006
Single Cell Cartridge		NC003
Human IgG PE-conjugated mAb		F0157
Human IgG ELISA Kit		NBP3-14790
Cells CHO-S Stable Pool (EL186)		In house
96-Well Plates	Corning	3595
Molecular Biology Grade Water		46-000-CM
Media		
ClonaCell™ CHO ACF Supplement	Stemcell	3820
Cytiva HyClone ™CDM4MAb™ Media	Fisher Scientific	SH3080202
Conditioned media 0.2 µm-filtered supernatants from wild-type CHO-S cell culture	N/A	N/A
Cytiva HyClone™ CDM4CHO™ Media	Fisher Scientific	SH3055802
Gibco™ GlutaMAX™ Supplement	Fisher Scientific	35 050 061
Anti-Clumping Agent	Thermo Fisher	0010057DG

TABLE 2. Materials used in this study.

IgG Titer Determination by ELISA

An 8-pt, 2-fold serial dilution series of Human IgG was prepared from 100 ng/mL to 1.5625 ng/mL to create a standard curve with a four-parameter logistic (4PL) regression. The cold-captured labeled and unlabeled clone cell supernatant was diluted 1:2,000 in ELISA Sample Diluent. The assay controls included wild-type CHO-S cells and CHO-S stable pool (EL186) cells, diluted to 1:2,000 in ELISA Sample Diluent. The blanks included the ELISA Sample Diluent and the complete cloning media, diluted to 1:2,000 in ELISA Sample Diluent. Due to ELISA space constraints, the cold capture labeled and unlabeled samples with low-density cells were limited to 4 for each. Wells that showed more than 1 colonies were excluded. In addition, several cold captured clones exhibited false negative ELISA results and were excluded from the IgG titer assessment summary (FIGURE 9).

Results of the Namocell Cold Capture Assay

We started with a CHO stable pool expressing the monoclonal antibody of interest. We then prepared two aliquots of the stable pool, one aliquot for cold capture labeling with PE-conjugated anti-human IgG antibody and the other aliquot remaining unlabeled. Once the labeling protocol described in the Materials and Methods was completed, we load the stained cells into the Namocell microfluidics cell cartridge and dispense the cold-captured cells into 96-well plates. Similarly, the unlabeled cells were loaded into a new cartridge and dispensed into 96-well plates. Both labeled and unlabeled cells were then allowed to culture for 3 weeks. Finally, an IgG titer was determined for all surviving clones using the ELISA assay.

The Namocell software allows for multiple, easy gating to rapidly isolate cells of interest. Forward scatter and side scatter were used to observe all cells, and the PE channel for fluorescent labeled cells, which allowed us to easily identify the brightest cells and deposit them as individual clones (FIGURE 6).

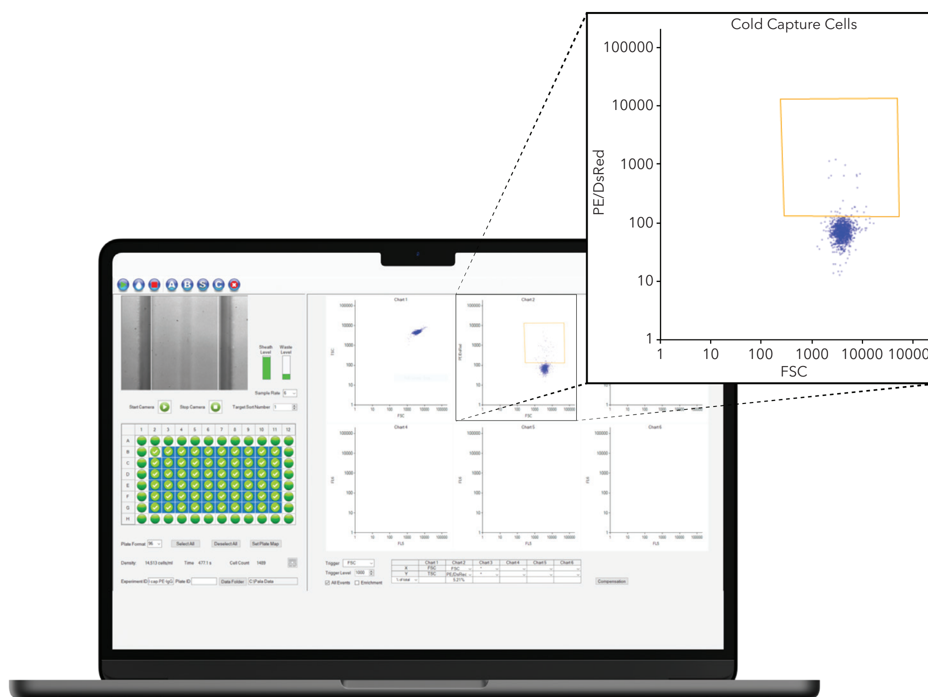


FIGURE 6. Screenshot of the Namocell software and gating of the top 5% brightest cells in the PE channel.

Cold capture analysis using the Pala Namocell yielded 3X more clones than the unlabeled method in both high- and low-density cells (FIGURE 7). Overall, 41 cold capture clones survived through culturing compared to only 14 unlabeled clones, confirming improved clone survival using the cold capture method as previously described in the cold capture studies.

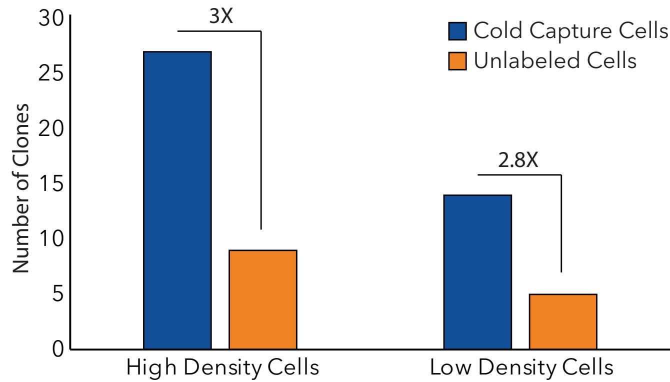


FIGURE 7. Cold capture analysis using the Pala Namocell. Percentage of cold capture labeled cells and unlabeled cells following 3 weeks of culture.

Once all surviving clones were identified, the supernatant was collected, and titer was determined for each clone using an anti-human IgG ELISA to identify the highest producers. The titers were determined using standard curves of the Human IgG serial dilution series with a weighted 4PL regression (FIGURE 8). The interpolated values and percent recovery for each point of the standard curves showed high recovery for each point in the standard curves (TABLE 3).

Standards (ng/mL)	Interpolated (ng/mL)	% Recovery
Plate 1		
100	108.7	108.7
50	47.6	95.2
25	23.5	94.1
12.5	13.3	106.8
6.25	6.5	104.3
3.125	2.8	89.4
1.5625	1.6	105.3
Plate 2		
100	108.7	108.7
50	47.6	95.2
25	23.5	94.1
12.5	13.3	106.8
6.25	6.5	104.3
3.125	2.8	89.4
1.5625	1.6	105.3

TABLE 3. Interpolated concentrations and percent recovery of the IgG standard curves. The IgG standard curves on ELISA were used to quantify IgG titers in cold capture labeled and unlabeled cells (1 plate each).

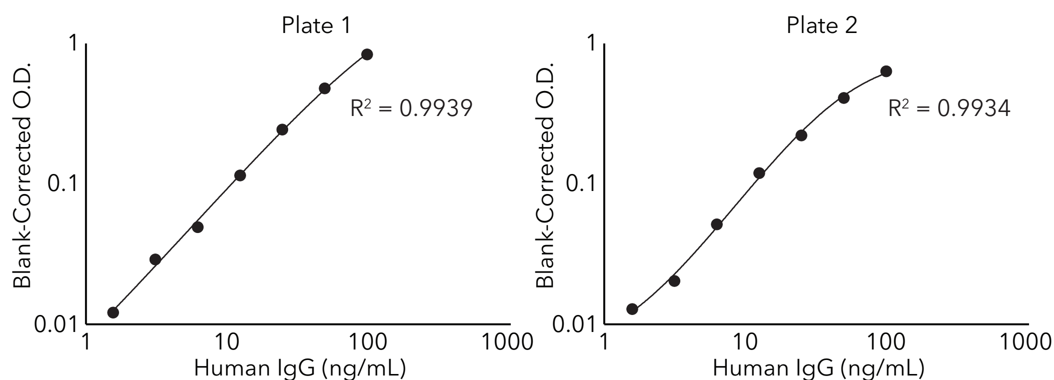


FIGURE 8. Human IgG standard curves on ELISA. 4PL weighted regression was used to quantify IgG titers in cold capture labeled and unlabeled cells (1 plate each).

IgG determination by ELISA showed that more than 44% of the cold capture clones produced at least 100 μg/mL of IgG compared to none for unlabeled clones (FIGURE 9), including two cold capture clones that produced more than 200 μg/mL IgG (TABLE 4).

These results clearly demonstrate that cold capture enriches for high titer IgG-producing clones. On average, cold capture clones produced 3X more IgG than the unlabeled clones and 4X more than the stable pool.

Sample	# of Clones	Avg. (μg/mL)	Range (μg/mL)
CHO-S Pool	N/A	25.7	20.5 - 31.0
Cold Captured	27	100.6	0 - 282.6
Unlabeled Cells	9	32.0	0 - 92.6

TABLE 4. The average and the range of IgG titer of CHO cells. Titers were measured from individual clones isolated with or without the cold capture technique or from the pool of CHO-S cells.

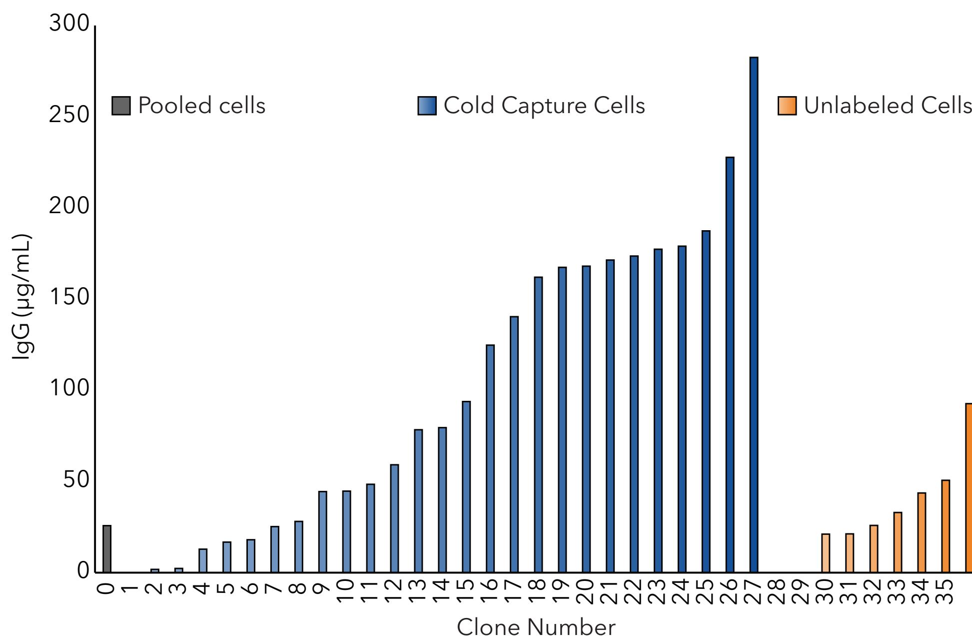


FIGURE 9. Cold capture enriches for high titer IgG-producing CHO cells. Comparison of IgG titer of CHO-S cell pool stably transfected with IgG (gray) and individual clones derived from the same pool with cold capture (blue) or without cold capture (orange).

Cold Capture with Namocell Pala Enriches for High Titer Clones

Biotherapeutics are complex drugs that require careful manufacturing. This manufacturing all starts with the cell. Cell line development, and specifically clone isolation and selection are one of the most critical steps to being successful. The most common techniques for single cell isolation are limiting dilution and FACS, each with its own challenges.

Here, we demonstrated the cold capture technique and how it can be combined with Namocell single cell sorting to enable more efficient identification of high-producing clones. The combination of the cold capture approach with Namocell enriches for the highest IgG-producing cells compared to unlabeled single cell dispensing, resulting in clones that produced on average 3X more IgG.

In addition, cold capture labeling with Namocell enriched for more robust clones than unlabeled single cell dispensing as can be observed in clone survival after 3 weeks of culturing. Together our data show that this new workflow is fast, efficient, and effective at generating robust and attractive cell clones for downstream use.

References

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