APPLICATION NOTE

Opto® Memory B Discovery Human Workflow

Unlock access to the wide diversity of human memory B cells to accelerate more physiologically relevant and efficacious antibody discovery.

In this Application Note we outline:

- A rapid workflow to screen human memory B cells and discover high-value monoclonal antibodies in one week
- Robust activation of human memory B cells using proprietary feeder-free activation medium
- Efficient recovery of antigen-specific antibody sequences for downstream characterization and development

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Introduction

Opto® Memory B Discovery Human on the Beacon® Optofluidic System enables rapid selection of lead molecules with function-forward screening of B cells from donor samples in under 1 week. While Opto® Plasma B Discovery workflows enable screening of primary plasma B cells, the Opto Memory B Discovery Human workflow enables rapid screening of human memory B cells.

Human antibodies have inherently lower immunogenicity without need for humanization when developing therapeutic antibodies, as the antibodies and targets are already physiologically relevant. Accessing the human repertoire for antibody discovery can thus accelerate the timeline toward safe and efficacious therapeutics, especially in an urgent emerging disease response[1] and controlling endemic diseases[2]. Beyond antibody therapeutics, directly screening human B cells opens opportunities to studying antibody diversity, prevalence, and population kinetics, giving insights into disease progression and humoral immunity that are especially important with vaccine[3] and auto-immunity research.

Single B cell screening approaches are attractive strategies for discovery of human monoclonal antibodies as compared with hybridoma and phage display owing to the low fusion efficiency and loss of cognate heavy/light chain pairing in the latter two approaches, respectively. A central challenge to single B cell screening in humans is the lack of robust B cell media to maintain durable B cell survival and antibody secretion. As a result, human B cell screening approaches are typically performed using memory B cells that must be activated to secrete antibodies for screening purposes.

Workflow Overview

Bruker's Opto Memory B Discovery Human workflow is an end-to-end workflow that enables robust feeder-free activation of human memory B cells, followed by on-chip assays and downstream sequence recovery (FIGURE 1).

The workflow's Opto® Memory B Discovery Sample Prep Kit, Human enables activation of memory B cell samples from fresh whole blood or frozen PBMC donor samples (FIGURE 1A and 1B). The Beacon system then automatically isolates tens of thousands of single activated B cells into NanoPen® chambers on OptoSelect® 11k or 20k chips in under 1 hour per chip (FIGURE 1C). Antigen-binding and cross-reactivity assays are used to screen and select activated B cells secreting antigen-specific antibodies (FIGURE 1D). Single antigen-specific B cells can then be recovered into 96-well plates (FIGURE 1E) followed by off-chip cDNA synthesis and amplification using the Opto® B Discovery cDNA Synthesis® Kit (FIGURE 1F). Paired antibody heavy/light chain sequences are amplified from the cDNA of recovered cells using the Opto® B Discovery Sanger Prep Kit, Human, which includes a primer for conventional Sanger sequencing (FIGURE 1G and 1H).

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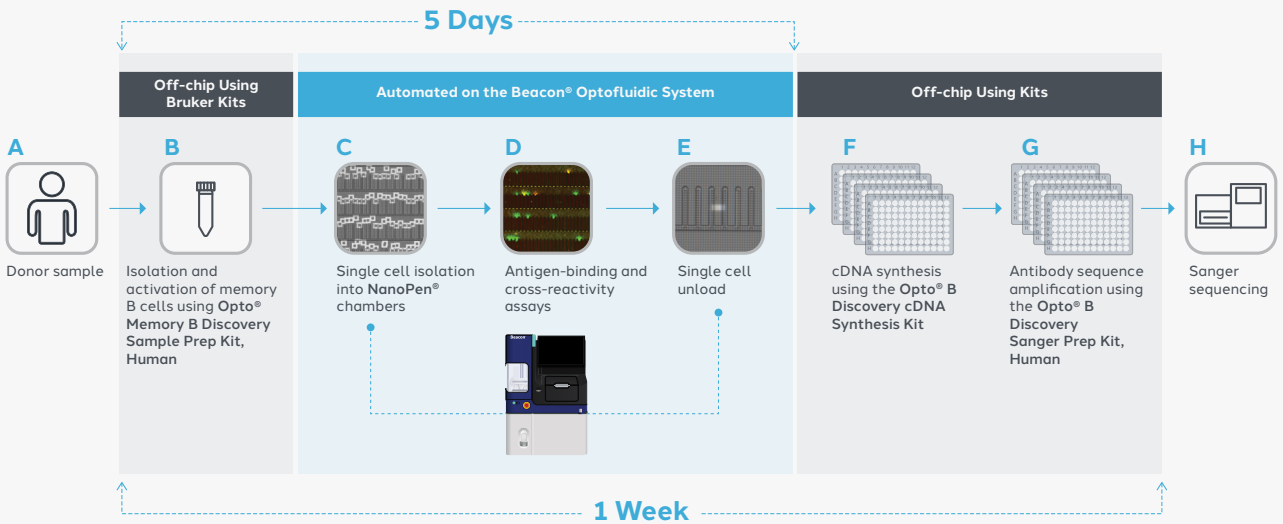

FIGURE 1: The Opto Memory B Discovery Human Workflow enables isolation and activation of memory B cells, function-forward single B cell screening, and recovery of human monoclonal antibody sequences.

Memory B Cell Isolation and Activation

The Opto Memory B Discovery workflow begins by either isolating peripheral blood mononuclear cells (PBMCs) from fresh whole blood, or by thawing frozen PBMCs. Memory B cells are then isolated from PBMCs via off-the-shelf MACS kits and activated for 5 days in culture using the Opto Memory B Discovery Sample Prep Kit, Human (FIGURE 2A). In 35 PBMC samples harvested from 5 vaccinated and/or convalescent donors the average frequency of memory B cells was 0.56% (range 0.15% - 1.5%, FIGURE 2B). Bruker's proprietary feeder-free Human Memory B Cell Activation Medium produces activated B cells with robust proliferation (2.6-31.6-fold, n=39 samples FIGURE 2C) and high viability (62-99%, n=48 samples, FIGURE 2D). After 5 days, activated B cells are ready for screening on the Beacon system.

The Opto Memory B Discovery workflow does not require antigen-positive sorting of memory B cells, thus enabling screening of difficult cell-based targets that cannot be recombinantly expressed as soluble molecules. However, in cases where the antigen-positive memory B cell populations are rare, standard enrichment strategies pre-activation and/or post-activation can be adapted to increase the likelihood of finding rare antibodies (dotted lines, FIGURE 2A).

Memory B Cell Isolation and Activation for Opto Memory B Discovery on Beacon

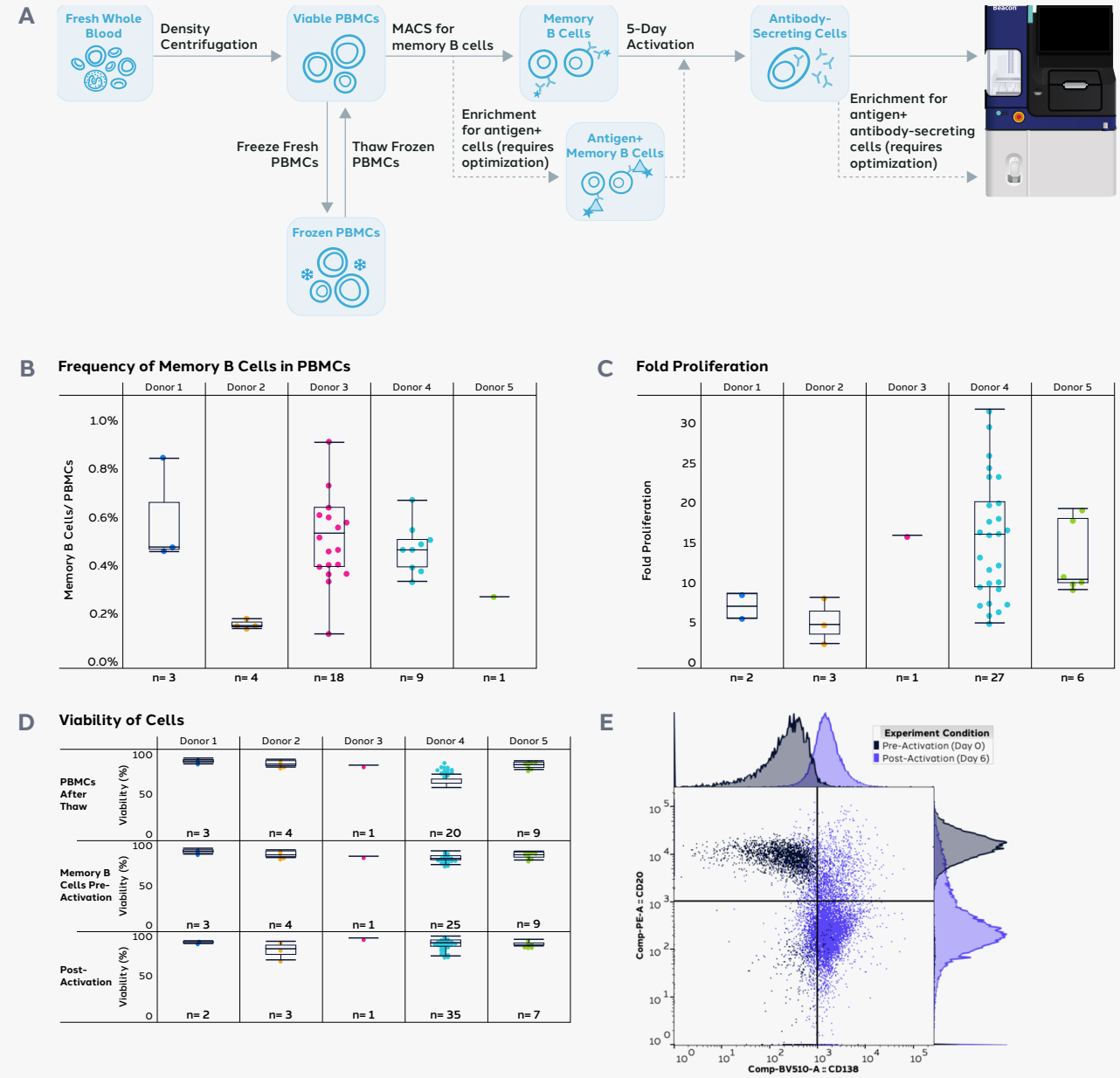

FIGURE 2: The Opto Memory B Discovery workflow isolates and activates memory B cells from human PBMC samples to produce antibody-secreting cells that are suitable for screening. A) Schematic of Activation Process. B) Frequency of IgG+ memory B cells in human PBMC samples. C) Fold-proliferation of human memory B cells post-activation. D) Viability of human PBMCs after thawing and memory B cells before and after activation E) The pre- vs. post-activation expression levels of CD20 and CD138 showing the differentiation of memory B cells into antibody-secreting plasma cells.

Single B Cell Isolation on the Beacon System

After 5 days of incubation, activated human B cells are loaded onto OptoSelect 11k or OptoSelect 20k chips and isolated as single B cells into NanoPen chambers. Over 7,500 or 12,500 single B cells can be loaded and screened per OptoSelect 11k chip or OptoSelect 20k chip, respectively. Workflows can use up to four chips at a single time, enabling a total single B cell screening throughput of 7,500 to up to 60,000 cells per workflow (FIGURE 3).

Flexible High Throughput Screening for Even the Rarest Antibodies

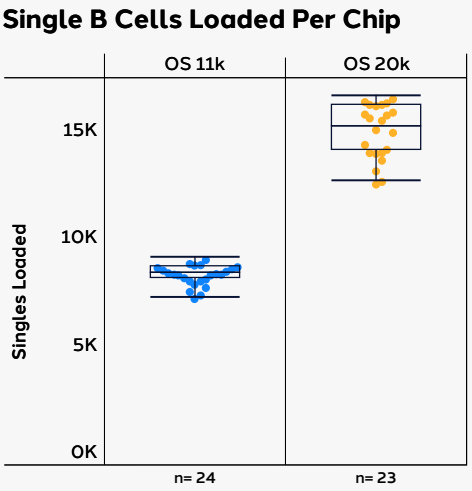

FIGURE 3: The Opto Memory B Discovery Human workflow enables screening over 7,500 to 60,000 single activated human B cells per workflow using OptoSelect 11k or 20k chips.

On-Chip Assays for Functional Profiling

Following loading, activated human B cells can be screened using multiple on-chip assays for antibody specificity and cross-reactivity. Cell Analysis Suite (CAS®) software uses machine learning to automatically score assays and identify NanoPen chambers that contain B cells secreting antigen-specific antibodies. Users can then manually verify assay results using Image Analyzer software.

Bead-based assays for IgG antibody secretion can be used to assess successful activation of memory B cells. From 16 workflows performed on samples from vaccinated and/or convalescent donor samples against SARS CoV-2, 39% of activated single B cells secreted IgG antibodies on average (FIGURE 4A). Bead-based assays can also be used to discover antigen-specific antibodies. In the same 16 workflows, an average of 0.18% of single B cells were positive for the soluble SARS-CoV-2 antigen (FIGURE 4B). The Human Memory B Cell Medium used in the workflow maintains an average of 86% of IgG secreting B cells 4 hours into the workflow (FIGURE 5), enabling more assays and longer, more complex assays to be run on precious donor samples.

As with previous Opto B Discovery workflows, on-chip assays for specificity against cell membrane antigens can also be performed by replacing beads with cells over-expressing the target antigen. This unlocks the ability to perform antibody discovery against more challenging membrane antigens that cannot be expressed solubly and are therefore incompatible with other technologies.

On-Chip Functional Profiling of Activated Human B Cells

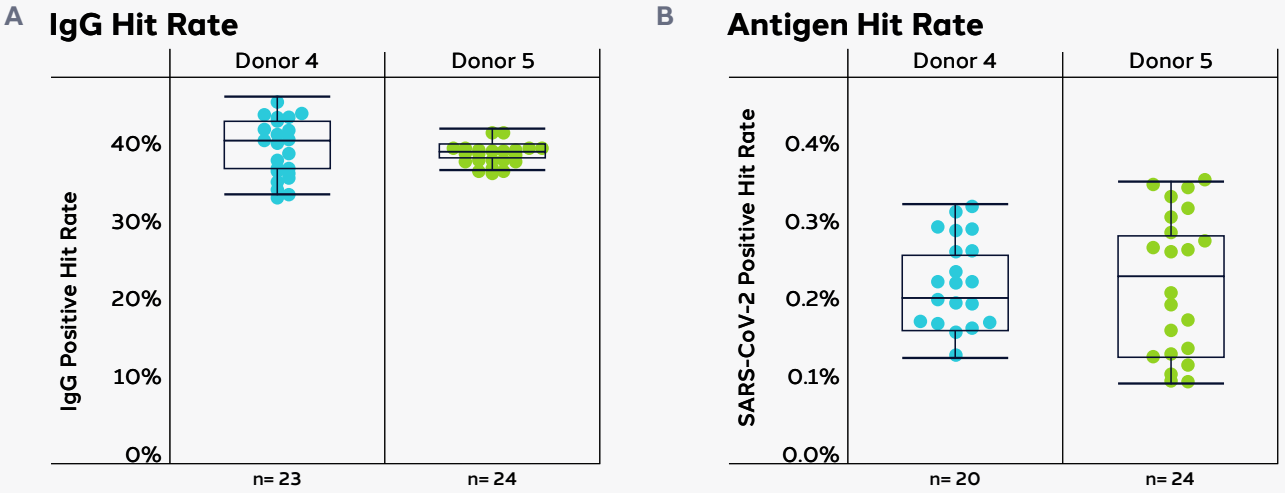

FIGURE 4: A) Fraction of activated B cells secreting IgG using PBMC samples from vaccinated or convalescent human donors against SARS-CoV-2. B) Fraction of activated B cells screened secreting SARS-CoV-2-specific antibodies from those donors.

Sequence Recovery

The Opto Memory B Discovery Human workflow recommends recovery of paired heavy/light chain sequences for antigen-specific antibodies via Single Cell Unload (FIGURE 6). This approach directly recovers single cells into 96-well plates to perform off-chip cell lysis, cDNA synthesis and amplification, and antibody heavy/light chain amplification (FIGURE 5B). This is the preferred method for high recovery rates of paired heavy/light chain sequences, especially when there are fewer than 192 hits.

The PCR products can then be sequenced using traditional Sanger Sequencing. On average, 75% of pens targeted for export yielded paired heavy/light chain sequences with unambiguous nucleotide bases across the entire V(D)J regions (FIGURE 7, Strict). These same samples yielded sequences that had no ambiguous bases across the CDR regions 78% of the time, on average (FIGURE 7, Moderate), and no ambiguous bases across the CDR3 region 80% of the time, on average (FIGURE 7, Relaxed). Sequences with ambiguous base calls may be recovered with traditional cloning and/or re-sequencing methods.

Interrogate More Deeply with More Assays and Longer B Cell Survival

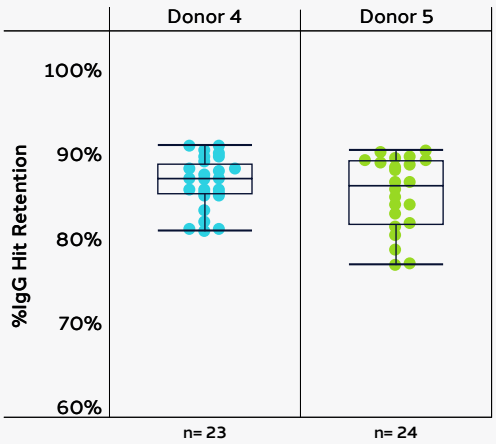

FIGURE 5: Fraction of activated human B cells that continue to secrete antibodies 4 hours after loading on chip as measured by bead-based assays for IgG secretion

Recover Heavy and Light Chain Antibody Sequences Directly with Single Cell Unload

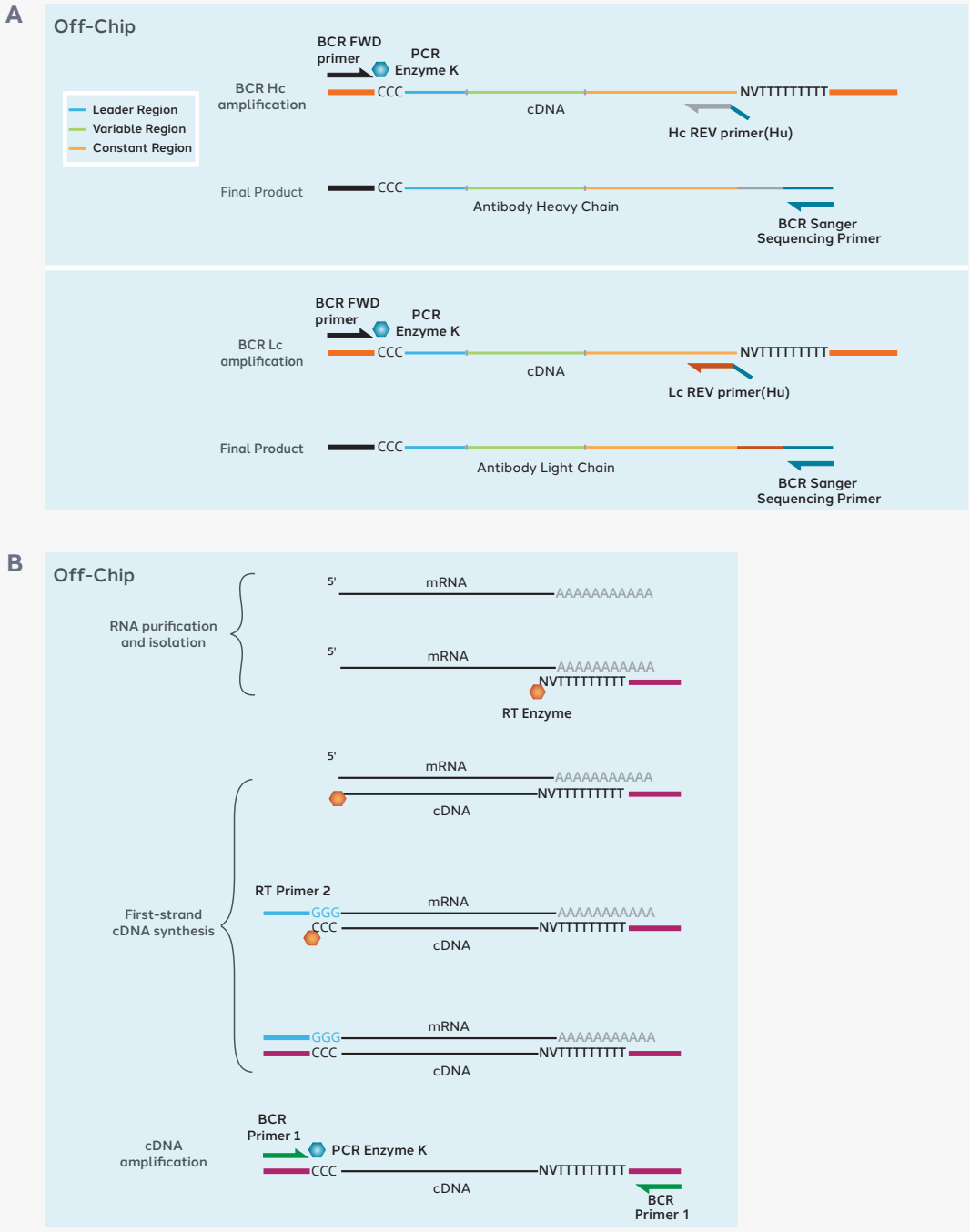

FIGURE 6: Antigen-specific antibody heavy/light chain (Hc/Lc) sequences can be obtained using Single Cell Unload. A) Using Single Cell Unload, all molecular biology steps (lysis, reverse transcription, cDNA synthesis and amplification) are performed after recovery of cells in 96-well plates. B) The amplified cDNA from Single Cell Unload is used for amplification of the antibody heavy/light chain genes by the Opto B Discovery Sanger Prep Kit (Human).

Recover More Valuable Sequences from the Opto Memory B Discovery Human Workflow

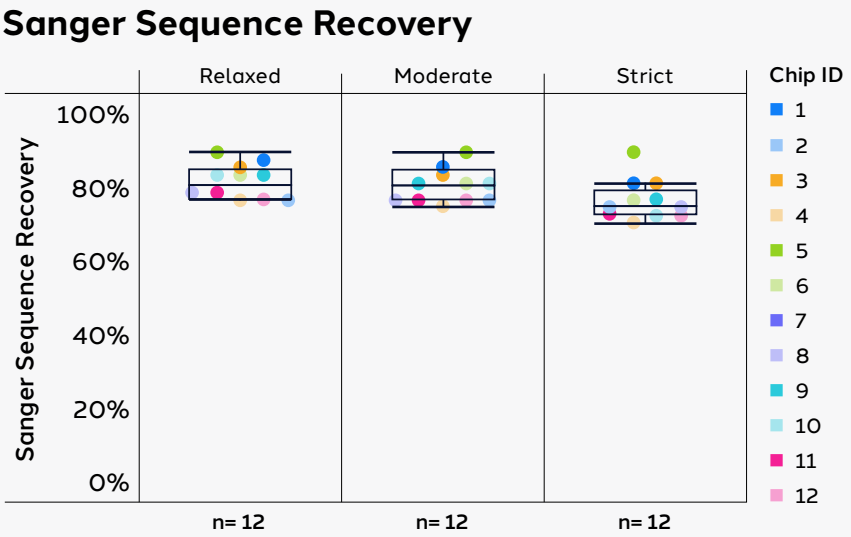

FIGURE 7: The Opto Memory B Discovery Human Workflow enables efficient recovery of antigen-specific antibody sequences for downstream re-expression and confirmation assays. Sequences can be recovered by unloading cells into well plates for Sanger sequencing. Data plotted from 12 chips with Cell Unloads against varying definitions of successful recovery. Relaxed: heavy and light chain sequences align in IgBlast and have no N nucleotide assignments in CDR3. Moderate: Relaxed and no N nucleotide assignments in any CDRs. Strict: Moderate and no N nucleotide assignments in the entire variable V(D)J domain.

References

1. Zost, S.J., Gilchuk, P., Chen, R.E. et al. Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. Nat Med 26, 1422–1427 (2020). https://doi.org/10.1038/s41591-020-0998-x

2. Hastie KM, Yu X, Ana-Sosa-Batiz F, et al. Potent Omicron neutralizing antibodies isolated from a patient vaccinated 6 months before Omicron emergence. Cell Rep. 2023;42(5):112421. doi: 10.1016/j.celrep.2023.112421.

3. Dacon, C., Tucker, C., Peng, L., Lee, C. C. D., Lin, T. H., Yuan, M., ... & Tan, J. (2022). Broadly neutralizing antibodies target the coronavirus fusion peptide. Science, 377(6607), 728-735.

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Order Information

Part Number	Product Name	Quantity (for up to 4 chips and 192 cell unloads)
750-08323	Opto Memory B Discovery Sample Prep Kit, Human • Loading Reagent (Part Number 750-08029) • Human Memory B Cell Activation and Culture Medium components (Basal Media and Additives) • Wetting Additive	1 (suitable for any number of workflows using up to 4 chips)
750-02030	Opto B Discovery cDNA Synthesis Kit	1 for each 192 Cell Unloads
750-08090 or 750-00019	OptoSelect Chip 11k or OptoSelect Chip 20k	1–4
750-08096	Import wells	1 (suitable for up to 25 x 4-chip workflows)
520-00053	Assay beads	1 (suitable for up to 16 assays with the OptoSelect 11k chip or 8 assays with the OptoSelect 20k chip)
750-02041	Opto B Discovery Sanger Prep Kit, Human	1 for each 192 Cell Unloads
750-08254 (optional)	OptoSeq Memory B Discovery PBMC Thawing Kit	1 for up to 4 vials of frozen PBMCs