DATASHEET
REV C | SEPTEMBER 2023

Antibody Discovery (AbD)

Opto® Memory B Discovery Rabbit Workflow

Key Highlights

- Rapid workflow to screen rabbit memory B cells to discover high-value monoclonal antibodies
- Robust activation of rabbit memory B cells using proprietary feeder-free activation media
- Efficient recovery of antigen-specific antibody sequences for downstream characterization and development

INTRODUCTION

Opto® Memory B Discovery on the Beacon® Optofluidic System enables rapid selection of lead molecules by function-forward screening of B cells from immunized animals in under 1 week. While Opto® Plasma B Discovery workflows enable screening of mouse plasma B cells, the Opto Memory B Discovery workflow enables rapid screening of rabbit memory B cells.

Rabbits have unique gene diversification strategies that produce antibodies with high affinity and specificity, making rabbit antibodies uniquely valuable reagents for basic research, diagnostics, and therapeutics development.[1] Rabbit polyclonal antibodies are widely used for research applications; however, monoclonal antibodies are often preferred for diagnostic and therapeutic applications due to their well-defined specificity and stability.

Single B cell approaches are an attractive approach for discovery of rabbit 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 rabbits is the lack of robust B cell markers that enable purification of rabbit plasma B cells. As a result, rabbit 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 Rabbit workflow is an end-to-end workflow that enables robust activation of rabbit memory B cells, followed by on-chip assays and downstream sequence recovery [**FIGURE 1**].

The workflow's Opto® Memory B Discovery Sample Prep Kit, Rabbit enables isolation and activation of memory B cell samples from fresh whole blood or frozen PBMC 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**]. Antigen-specific antibody sequences can then be recovered using the OptoSeq® BCR v2 kit. Automated cell lysis and reverse transcription are performed on-chip [**FIGURE 1E**] to generate stable cDNA on OptoSeq BCR mRNA capture beads. Amplification of cDNA is performed after recovering OptoSeq BCR beads into 96-well plates by exporting each bead to a distinct well [**FIGURE 1F**]. The Extended OptoSeq BCR Bead Unload Kit enables recovery of an additional 192 OptoSeq BCR bead exports [**FIGURE 1G**]. Alternatively, single antigen-specific B cells can be recovered into 96-well plates [**FIGURE 1H**] followed by off-chip cDNA synthesis and amplification using the Opto® B Discovery cDNA Synthesis Kit [**FIGURE 1I**]. Paired antibody heavy/light chain sequences are amplified from the cDNA of recovered beads or cells using the Opto® B Discovery Sanger Prep Kit, Rabbit, which includes a primer for conventional Sanger sequencing [**FIGURE 1J and 1K**].

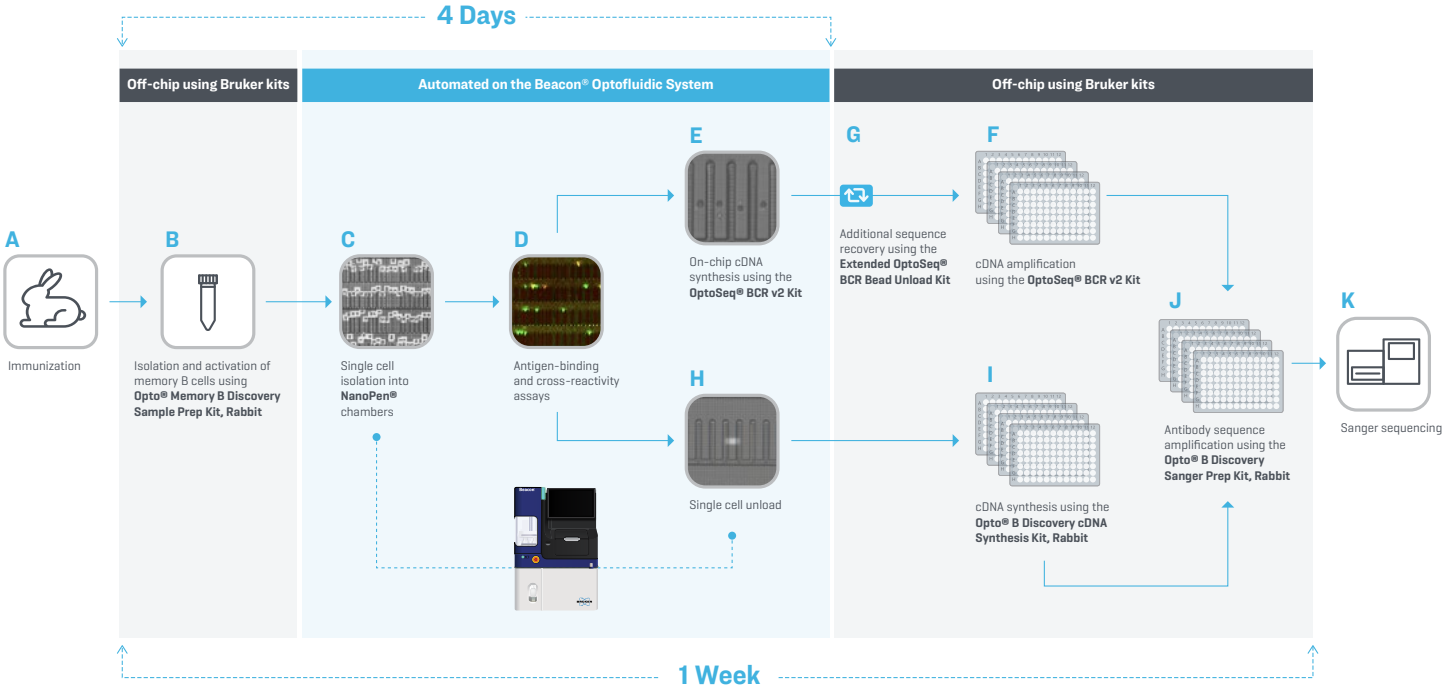

FIGURE 1: The Opto Memory B Discovery Rabbit Workflow enables isolation and activation of memory B cells, function-forward single B cell screening, and recovery of rabbit monoclonal antibody sequences.

MEMORY B CELL ISOLATION AND ACTIVATION

The Opto Memory B Discovery workflow begins by either isolating peripheral blood mononuclear cells (PBMCs) from fresh whole rabbit blood, or by thawing frozen rabbit PBMCs. IgG+ memory B cells are then isolated from PBMCs via MACS and activated for 4 days in culture using the Opto Memory B Discovery Sample Prep Kit, Rabbit (**FIGURE 2A**). In 13 PBMC samples harvested from unimmunized (naive) and immunized rabbits, the average frequency of IgG+ memory B cells was 2.1% (range 0.8% - 3.6%, **FIGURE 2B**). Bruker's proprietary feeder-free Rabbit Memory B Cell Activation Media produces activated B cells with robust proliferation (6-19x, n = 13 PBMC samples, **FIGURE 2C**) and high viability (76-98%, n = 13 PBMC samples, **FIGURE 2D**). After 4 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 proteins.

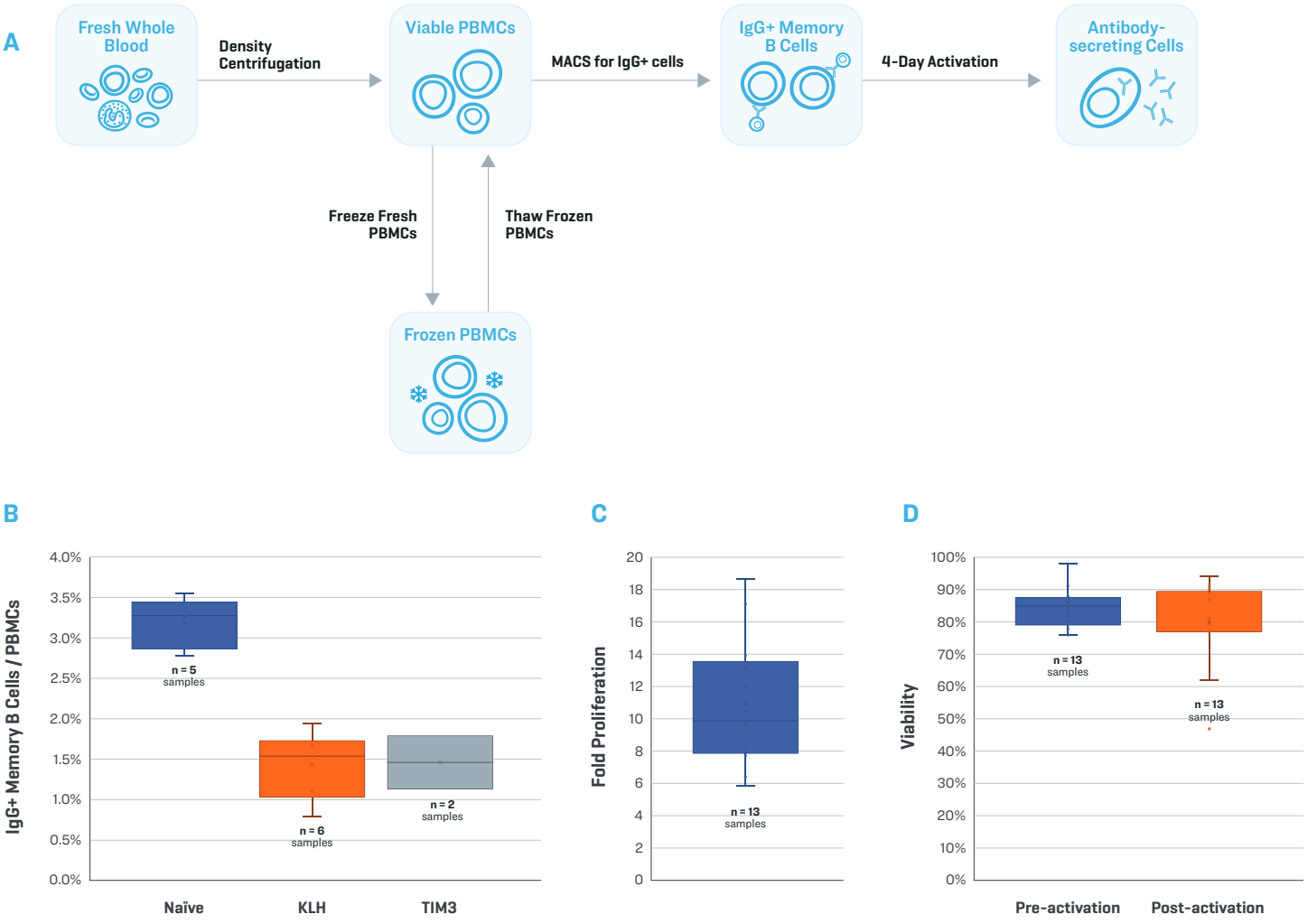

FIGURE 2: The Opto Memory B Discovery workflow isolates and activates memory B cells from rabbit PBMC samples to produce antibody-secreting cells that are suitable for screening. **A)** Schematic of Activation Process. **B)** Frequency of IgG+ memory B cells in rabbit PBMC samples. **C)** Fold-proliferation of rabbit memory B cells post-activation. **D)** Viability of rabbit memory B cells before and after activation.

SINGLE B CELL ISOLATION ON THE BEACON SYSTEM

After 4 days of incubation, activated rabbit B cells are loaded onto OptoSelect 11k or OptoSelect 20k chips and isolated as single B cells into NanoPen chambers. Over 15,000 to 30,000 single B cells can be screened using workflows with either 2 or 4 OptoSelect 11k chips. Alternatively, over 30,000 to 60,000 single B cells can be screened using workflows with either 2 or 4 OptoSelect 20k chips [FIGURE 3].

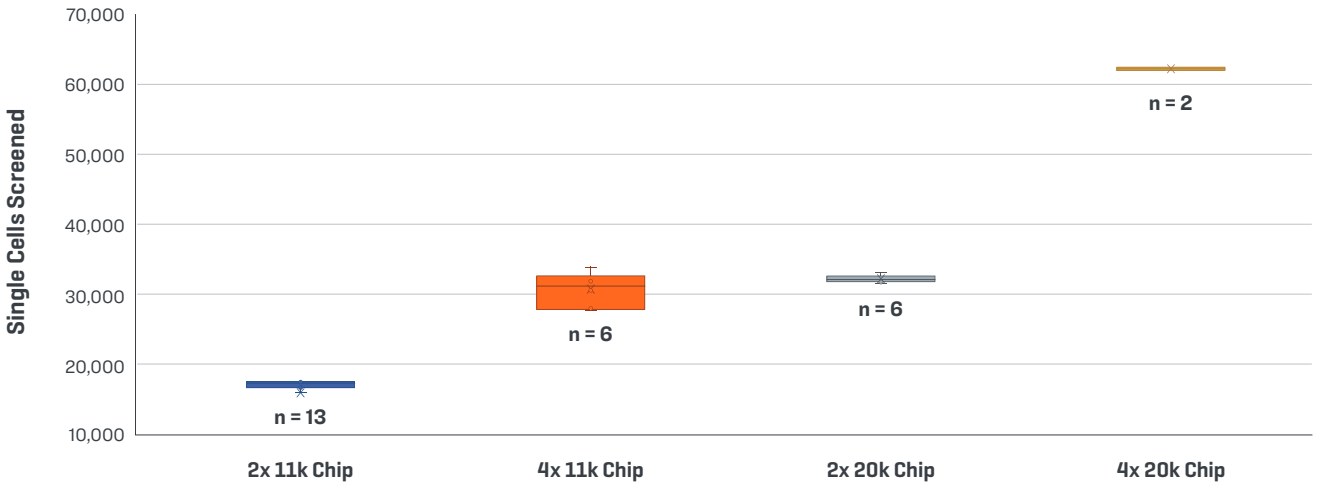

FIGURE 3: The Opto Memory B Discovery Rabbit workflow enables screening over 15,000 to 60,000 single activated rabbit B cells using OptoSelect 11k or 20k chips.

ON-CHIP ASSAYS FOR FUNCTIONAL PROFILING

Following loading, activated rabbit 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 26 workflows performed on samples from naive, unimmunized rabbits as well as rabbits immunized with soluble antigens (KLH or TIM3), the majority (>60% on average) of activated single B cells secreted IgG antibodies (**FIGURE 4A**). Bead-based assays can also be used to discover antigen-specific antibodies. From rabbits immunized with KLH, 10.6% of activated B cells secreted antigen-specific antibodies (average over n = 14 workflows), yielding between 388 and 5,821 antigen-positive hits per workflow depending upon the chip type and number of chips used (**FIGURE 4B**). From rabbits immunized with TIM3, 4.2% of activated B cells secreted antigen-specific antibodies (average over n = 6 workflows), yielding between 1202 and 2584 antigen-positive hits per workflow (data not shown). On average, over 85% of activated B cells continue to secrete antibodies after 4 hours of assays, enabling multiple assays to be performed for functional profiling (**FIGURE 4C**).

On-chip assays for specificity against cell membrane antigens can also be performed by replacing beads with cells over-expressing the target antigen.

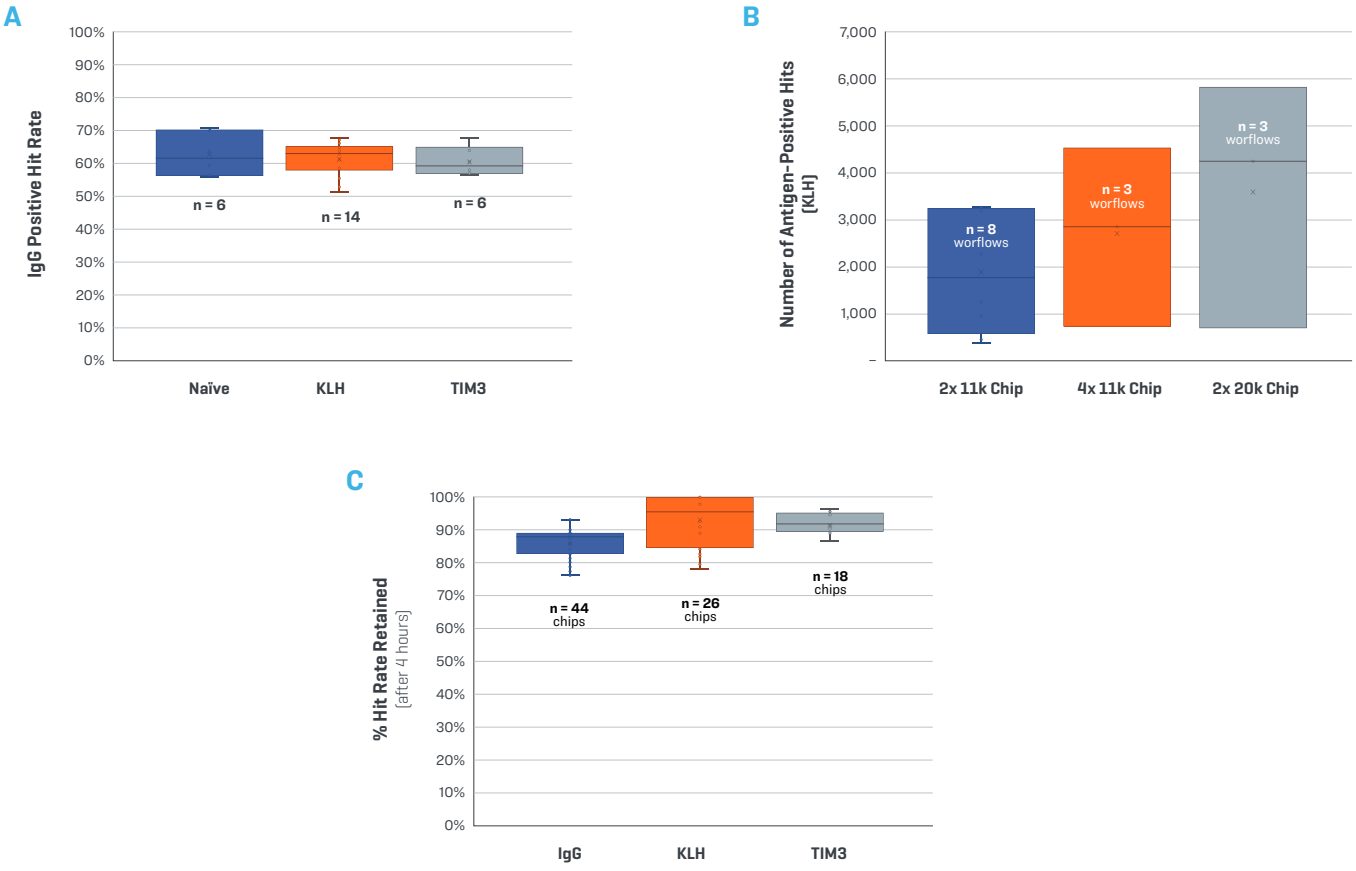

FIGURE 4: On-Chip Assays Enable Functional Profiling of Activated Rabbit B Cells. A) Fraction of activated B cells secreting IgG using PBMC samples from unimmunized (naïve) rabbits and rabbits immunized with two different antigens (KLH and TIM3). **B)** Number of hits discovered by screening activated memory B cells from KLH-immunized rabbits using varying numbers of OptoSelect 11k or 20k chips. **C)** Fraction of activated rabbit B cells that continue to secrete antibodies after 4 hours of assays as measured by bead-based assays for IgG secretion and antigen specificity.

SEQUENCE RECOVERY

The Opto Memory B Discovery workflow provides two approaches for recovery of paired heavy/light chain sequences for antigen-specific antibodies [FIGURE 5]:

1. **OptoSeq BCR Bead Approach:** On-chip cell lysis, mRNA bead capture, and cDNA synthesis followed by bead recovery into 96-well plates for off-chip amplification of cDNA and antibody heavy/light chain sequences [FIGURE 5A and 5C]; or,
2. **Single Cell Approach:** Recovery of single cells into 96-well plates to perform off-chip cell lysis, cDNA synthesis and amplification, and antibody heavy/light chain amplification [FIGURE 5B and 5C].

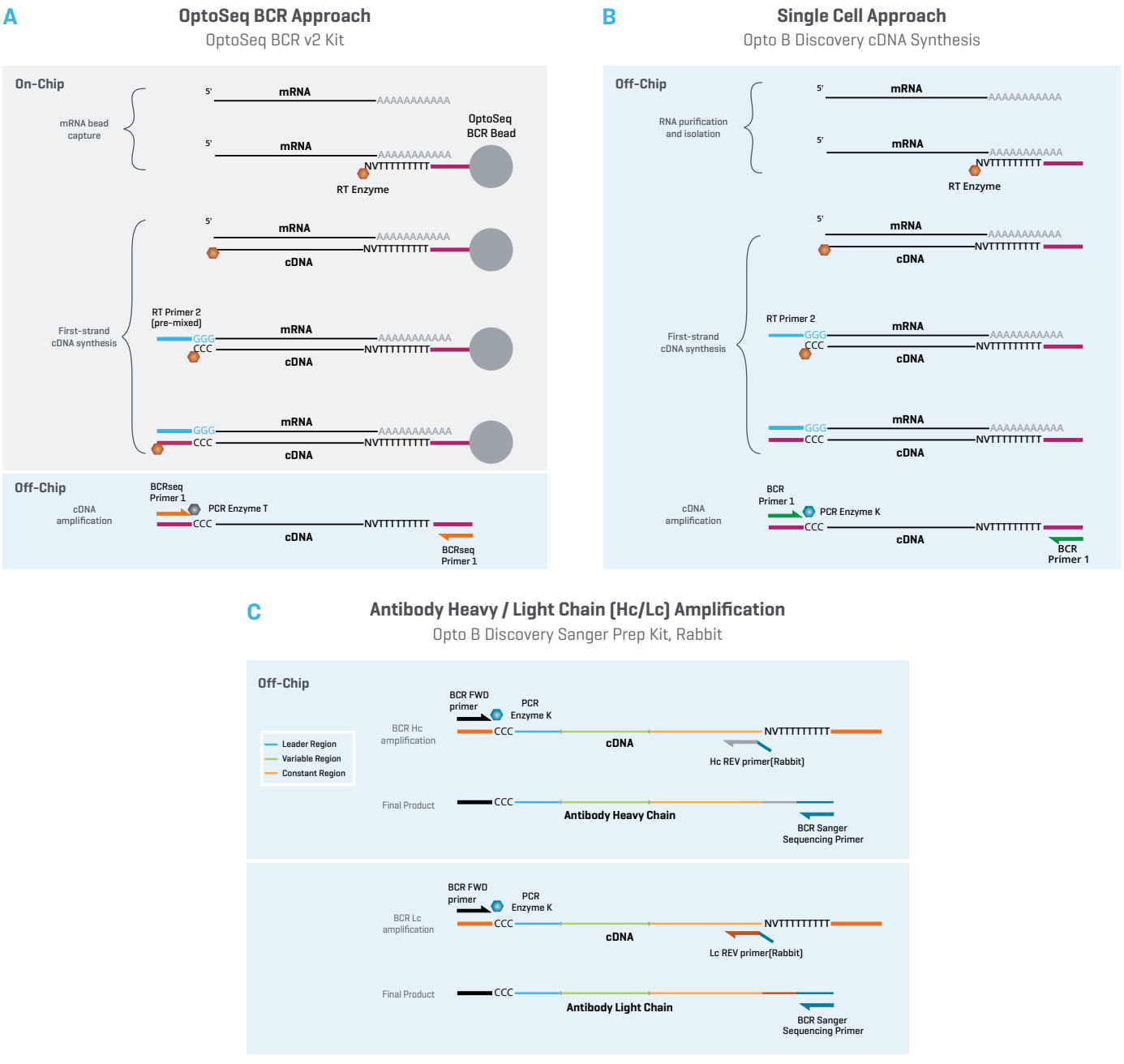

FIGURE 5: Antigen-specific antibody heavy/light chain (Hc/Lc) sequences can be obtained using either the OptoSeq BCR Bead Approach or the Single Cell Approach. **A)** Using the OptoSeq BCR bead approach, the OptoSeq BCR kit is used for on-chip cell lysis, on-chip cDNA synthesis and off-chip cDNA amplification from recovered beads. **B)** Using the Single Cell Approach, all molecular biology steps (lysis, reverse transcription, cDNA synthesis and amplification) are performed after recovery of cells in 96-well plates. **C)** The amplified cDNA from either the OptoSeq BCR Bead Approach or the Single Cell Approach is used for amplification of the antibody heavy/light chain genes by the Opto B Discovery Sanger Prep Kit (Rabbit). This figure depicts cDNA generated by OptoSeq BCR.

The PCR products of either OptoSeq BCR or Single Cell unloads can be sequenced using traditional Sanger sequencing. From OptoSeq BCR and Single Cell unloads respectively, on average 52% and 65% of pens targeted for export yielded paired heavy/light chain sequences with unambiguous nucleotide sequences (number of chips using OptoSeq BCR unload = 20, number of chips using cell unload = 20). The overall sequence recovery from both unload methods was 62% (**FIGURE 6**).

From over 1000 paired antibody sequences processed using a custom re-expression protocol, >89% of re-expressed antibodies (1090 out of 1230) yielded the expected antigen specificity as measured by ELISA.

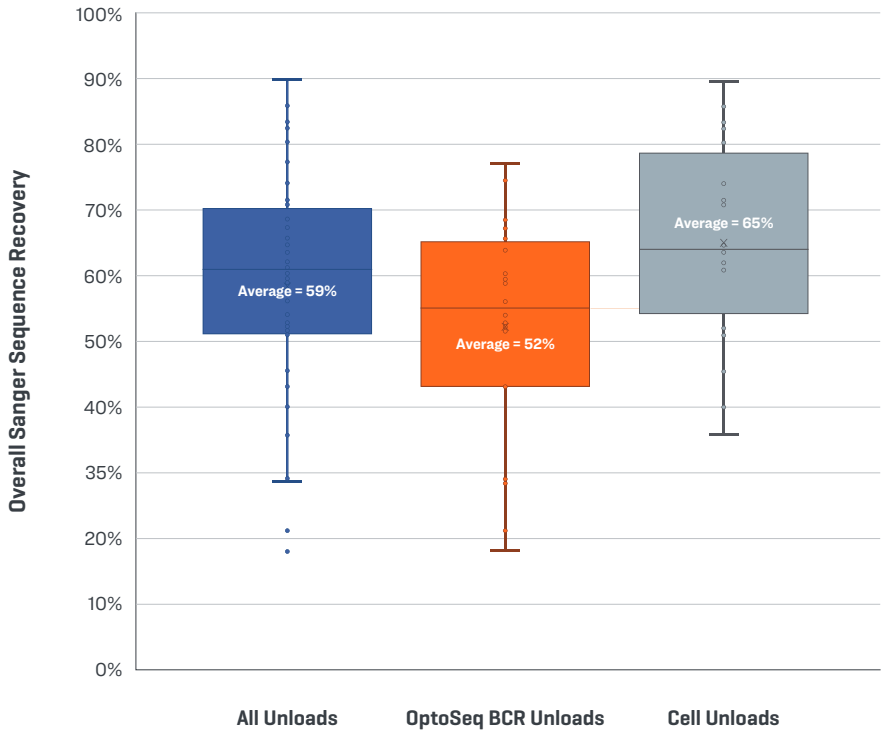

FIGURE 6: The Opto Memory B Discovery Rabbit Workflow enables efficient recovery of antigen-specific antibody sequences for downstream re-expression and confirmation assays. Sequences can be recovered by either unloading cells or OptoSeq BCR beads into well plates for Sanger sequencing. Data plotted from 20 chips with OptoSeq BCR unloads and 20 chips with Cell Unloads.

References

1 Zhang Z, Liu H, Guan Q, Wang L and Yuan H (2017), Advances in the Isolation of Specific Monoclonal Rabbit Antibodies. Front. Immunol. 8:494. doi: 10.3389/fimmu.2017.00494 Experimental & Molecular Medicine (2017) 49, e305; doi:10.1038/emm.2017.23; published online 24 March 2017.

ORDERING INFORMATION

PART NO.	PRODUCT NAME	QUANTITY (for up to 4 chips and 192 cell or OptoSeq BCR unloads)
750-08269	**Opto Memory B Discovery Kit, Rabbit** • Opto Memory B Discovery Sample Prep Kit, Rabbit • Opto Memory B Discovery PBMC Thawing Kit • Opto B Discovery Sanger Prep Kit, Rabbit	1 (suitable for any number of workflows using up to 4 chips and up to 192 OptoSeq BCR or Cell Unloads)
750-08251	**Opto Memory B Discovery Sample Prep Kit, Rabbit** • Memory B Cell Isolation Antibody, Rabbit • Loading Reagent (Part Number 750-08029) • Rabbit Memory B Cell Activation Media (Basal Media and Additives) • Wetting Additive	1 (suitable for any number of workflows using up to 4 chips)
750-08238 *or* **750-02030**	**OptoSeq BCR v2 Kit** *or* **Opto B Discovery cDNA Synthesis Kit**	1 for each 192 OptoSeq BCR Unloads or 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)
750-08257	**Opto B Discovery Sanger Prep Kit, Rabbit**	1 for each 192 OptoSeq BCR or Cell Unloads
750-08254 (optional)	**OptoSeq Memory B Discovery PBMC Thawing Kit**	1 for up to 4 vials of frozen PBMCs
750-08097 (optional)	**Extended OptoSeq BCR Bead Unload Kit**	1 for each 192 additional OptoSeq BCR beads

The Opto Memory B Discovery workflow is available on all Beacon systems with Import Well Nest Lids, bubble dislodge capabilities, and CAS 2.4.11.31+ installation.

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