The Opto® Antigen Presenting Bead Kit

The Opto® Antigen Presenting Bead (Opto APB) Kit, HLA*02:01, used in conjunction with multiple functional assays within the Opto® T Cell Profiling workflow on the Lightning® and Beacon® systems, makes it possible to link peptide-HLA binding and recognition to antigen-specific effector function. The kit allows users to load peptides of choice onto beads (**Figure 1**) and use them to assay peptide-HLA binding and stability, and to efficiently stimulate and expand antigen-specific T cells.

KIT OVERVIEW

The Opto® APB kit addresses a critical bottleneck in the tumor antigen discovery process – immunogenicity validation. The kit can be used to measure binding of putative antigenic peptides to HLA Class I (**Figure 2**), measure stability of peptide-HLA complexes over time (**Figure 3**), and, in conjunction with costimulatory antibodies pre-conjugated to the APBs, efficiently stimulate and expand endogenous antigen-specific T cells (**Figure 4**). In this manner, the Opto APBs can replace donor-derived antigen-presenting cells, removing the inherent variability and lack of quality control associated with cell-based antigen presentation. Data is shown from experiments in which a peptide from the melanoma-associated tumor antigen, SLC45A2, is used to stimulate T cells[1].

PEPTIDE-HLA BINDING

The peptide-HLA binding assay allows users to validate that a putative antigenic peptide binds HLA (**Figure 2A**). The HLA complex comes pre-loaded with an in-place peptide conjugated with FITC. This peptide-HLA complex goes into an exchange reaction with a user-provided

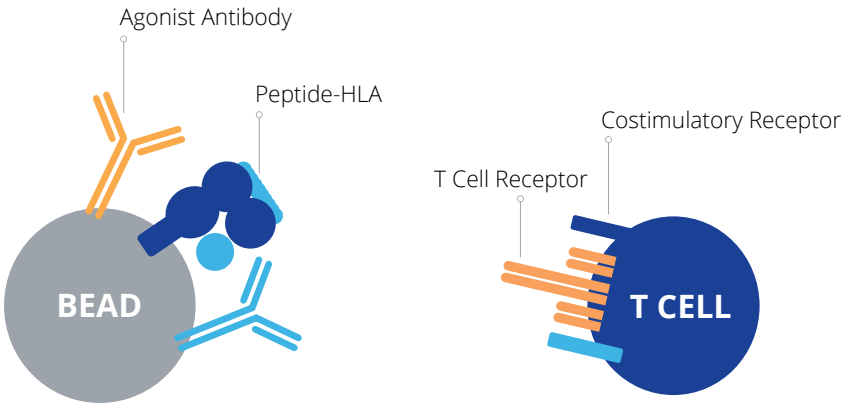

Figure 1. The Opto APBs allow scientists to measure peptide-HLA binding and stability, and efficiently stimulate and expand antigen-specific T cells.

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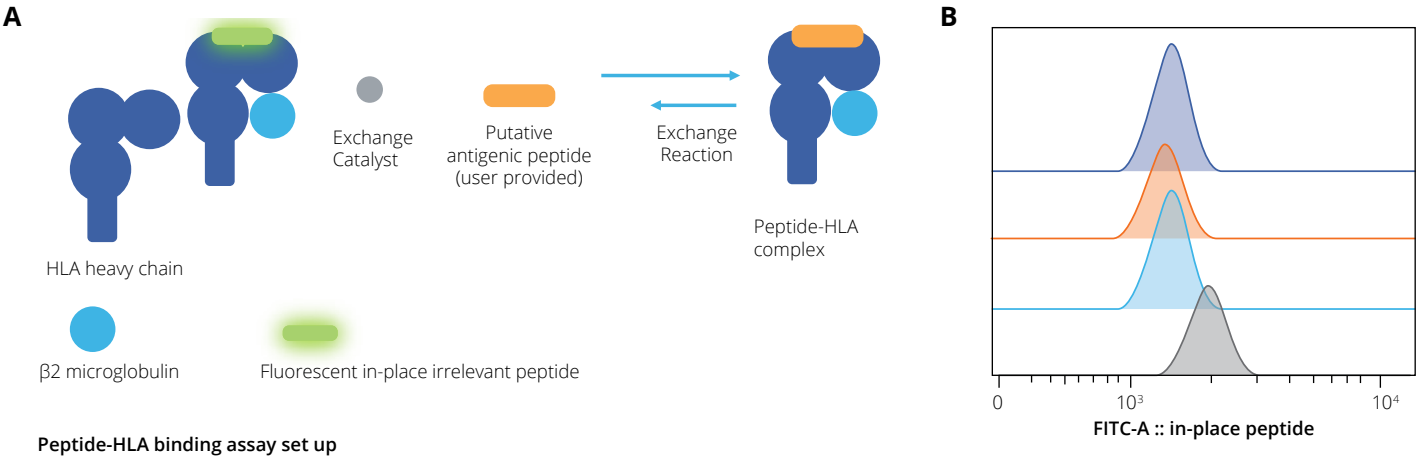

Figure 2. Representative results from the peptide-HLA binding assay. A. The peptide-HLA binding assay enables users to measure HLA binding of their putative antigenic peptide by competing out the fluorescent in-place peptide. **B.** Binding of the user-provided peptide to HLA is assessed by measuring a loss of FITC signal, as the fluorescent in-place peptide is displaced during the exchange reaction. Data is shown for beads without peptide-HLA bound (dark blue), beads without peptide exchange (light gray), beads in which control peptide has been loaded (orange), and beads in which SLC45A2 peptide has been loaded (blue).

peptide and flow cytometry is used to measure the degree to which the user-provided peptide can exchange out the in-place peptide. A decrease in FITC signal indicates that the in-place peptide has been displaced by the peptide (Figure 2B).

PEPTIDE-HLA STABILITY

Peptide-HLA Class I stability is a better predictor of CD8+ T cell immunogenicity than peptide affinity[2]. To assay peptide-HLA stability using this kit, APBs are prepared and incubated in T cell media at 37°C for a time course

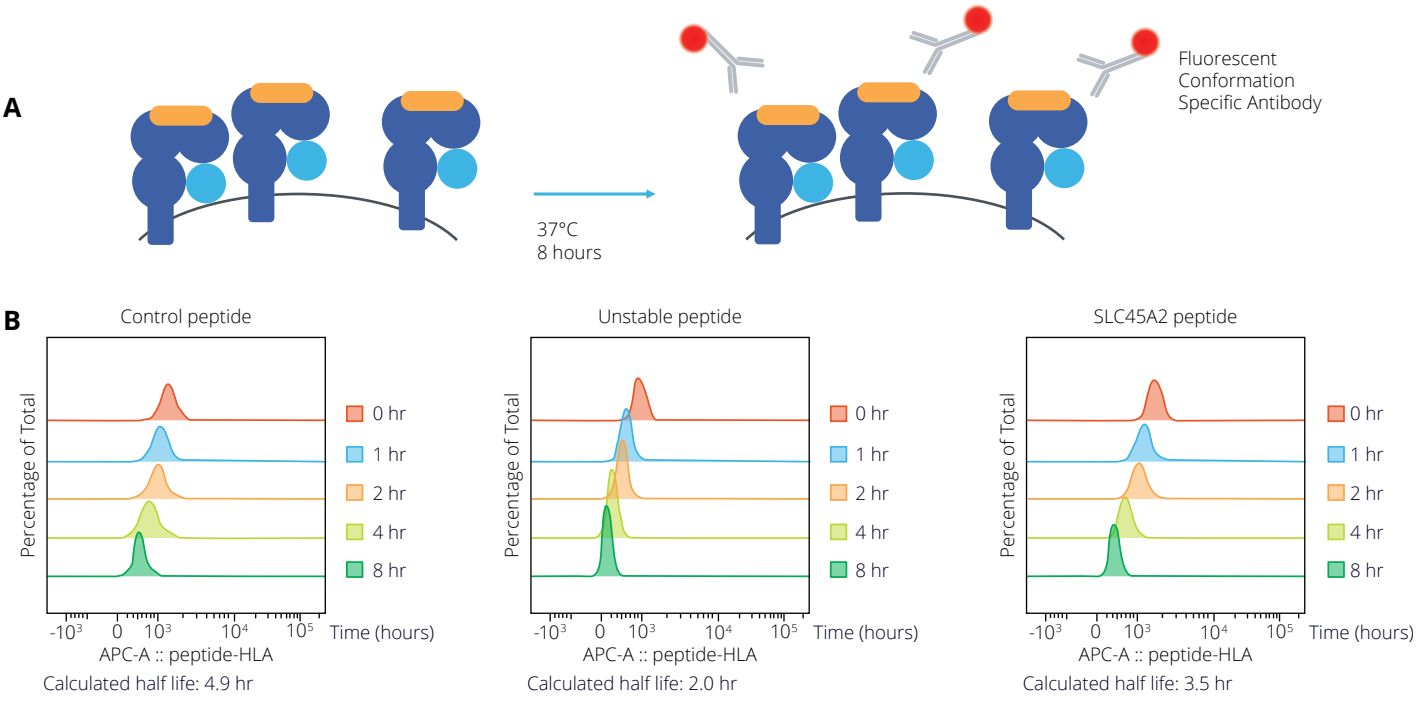

Figure 3. Representative results from the peptide-HLA stability assay. A. The peptide-HLA stability assay enables users to measure stable peptide-HLA binding by detecting intact HLA complexes over time. **B.** Results are shown for three peptides: control peptide (left), an unstable peptide derived from the tumor associated antigen TCL1 (middle), and the SLC45A2 peptide (right).

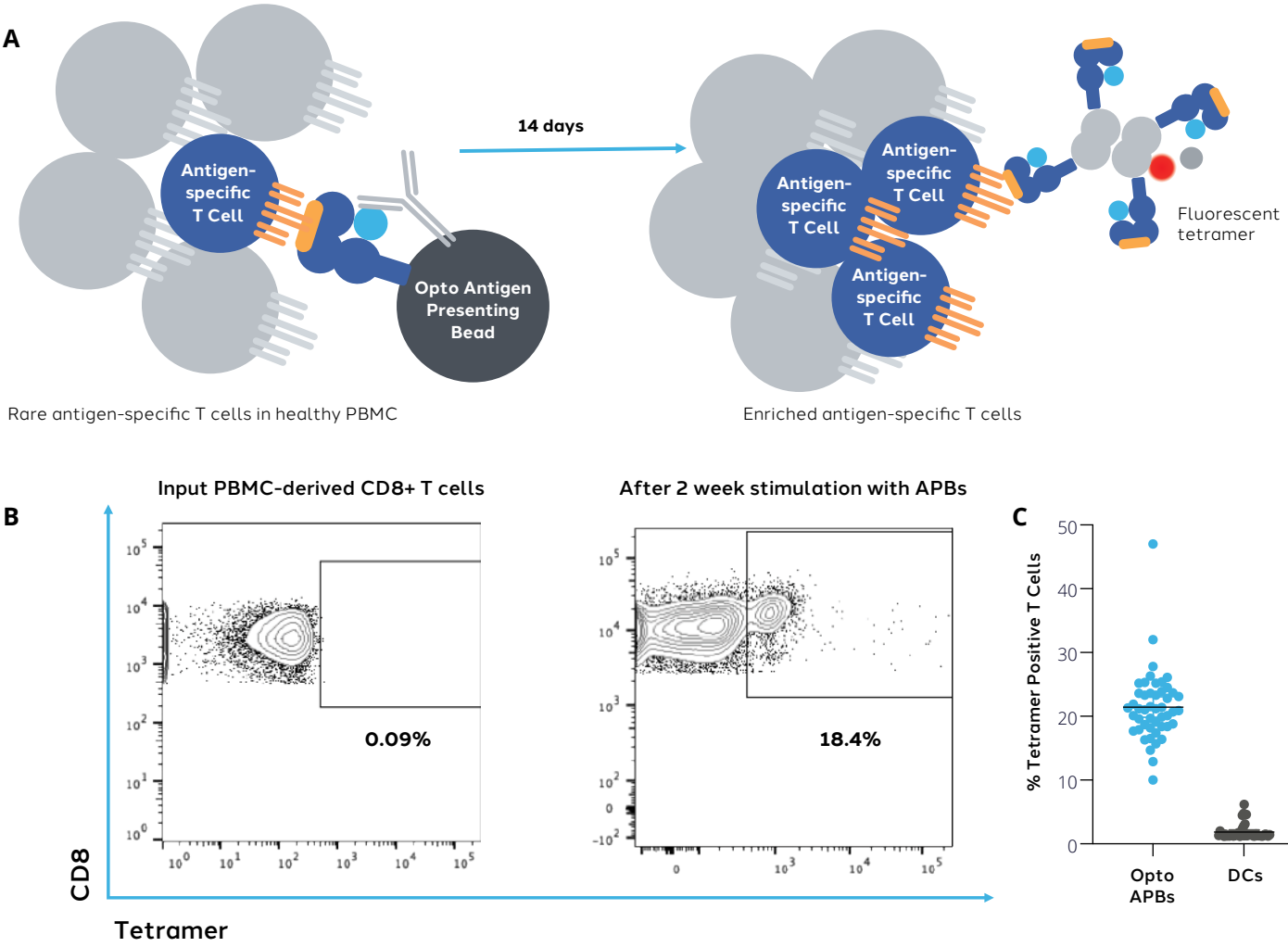

Figure 4. Antigen-specific T cell expansion. A. In only two weeks, Opto APBs can efficiently expand antigen-specific T cells. **B.** Flow cytometric analysis of antigen-specific CD8+ tetramer+ populations in input PBMC-derived CD8 cells (left) as well as after two weeks of stimulation with Opto APBs (right) shows robust expansion of SLC45A2-specific T cells. **C.** Opto APBs expanded significantly more SLC45A2-specific T cells than DCs.

of 8 hours (Figure 3A). APBs are harvested at 5 time points and intact, folded HLA complexes are quantified by flow cytometry (Figure 3B). Representative stability measurements are shown for the control peptide, an unstable peptide, and the stable SLC45A2 peptide[3] (Figure 3B).

ANTIGEN-SPECIFIC T CELL EXPANSION

The Opto APB kit is also capable of rapidly expanding rare endogenous, antigen-specific T cells from healthy donor peripheral blood more efficiently than dendritic cells (DCs) (Figure 4A). After measuring the capacity of SLC45A2 peptide to bind and maintain a stable peptide-HLA complex, we expanded T cells reactive to SLC45A2 using either Opto APBs or monocyte-derived dendritic cells. Antigen-specific T cells are detected using a fluorescently labeled tetramer, which can be purchased directly from MBL International.

Flow cytometry is used to analyze the frequency of SLC45A2-specific T cells both in PBMC-derived input CD8 cells as well as after two weeks of stimulation with Opto APBs (Figure 4B). When compared to cells stimulated with peptide-pulsed monocyte-derived DCs, the Opto APBs

Opto® T Cell Profiling Workflow

expanded SLC45A2-specific T cells approximately 10-fold more efficiently than DCs (Figure 4C).

CONCLUSION

Antigen-specific T cells can be selectively expanded using the Opto Antigen Presenting Bead kit upstream of the Opto Cell Therapy Development workflow, enabling you to directly link peptide binding and recognition to antigen specific effector function. Validating immunogenicity with this method will introduce a high degree of quality control and avoid the need to screen many peptides per protein target in order to identify only a few bona fide antigens[3].

REFERENCES

1. Bonsack et al., *Cancer Immunol Research*, 10 (2019): 2326–6066
2. Harndahl et a.l, *Eur Journal of Immunol*, 42 (2012)
3. Weng et al., *Blood*, 120 (2012): 1613–23

OPTO ANTIGEN PRESENTING BEADS KIT CONTENTS (750-01002)

PRODUCT NAME	PART NUMBER	COMPONENT	QUANTITY	VOLUME
Opto™ Antigen Presenting Beads Kit, HLA*02:01, Reagent Kit 1; Storage 4°C	520-02000	Antibody-Coated Beads	1	3.0 mL
	520-02001	Switchable pHLA*02:01	1	30 µL
	520-02004	APC Anti-HLA Antibody (HLA*02:01)	1	20 µL
	520-02005	Assay Buffer	1	30 mL
Opto Antigen Presenting Bead Kit, HLA*02:01, Reagent Kit 2; Storage 4°C	520-02003	Exchange Catalyst (HLA*02:01)	1	15 µL
	520-02006	Control Peptide (HLA*02:01)	1	15 µL

*The Opto® Antigen Presenting Bead Kit, HLA*02:01 is not available for purchase in Germany and the United Kingdom.*

REAGENTS MANUFACTURED BY OTHER VENDORS

PRODUCT NAME	PART NUMBER	COMPONENT	QUANTITY	VOLUME
HLA-A*02:01 - PE Tetramer for APB Kit	TB-T07300-1-BL	PE Switchable Tetramer (HLA*02:01)	3	0.5 mL

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brukercellularanalysis.com

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5858 Horton Street | Suite 320 | Emeryville, CA 94608

i+1-510-858-2855
brukercellularanalysis.com