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WHITE PAPER:

Efficient Hybridoma Screening with FlexAble Antibody Labeling Kits

Introduction

Monoclonal antibodies are vital tools in research, diagnostics, and therapeutics and demand for them continues to grow in various fields including cancer therapy and infectious disease diagnostics. This growing demand drives continuous research and innovation in antibody production technologies. While recombinant technologies that generate monoclonal antibodies have become broadly adopted, traditional hybridoma technology remains widely used. Over the years, advancements in cell culture techniques, media formulations, and cell engineering have improved hybridoma production efficiency and yield. We endeavor to show an additional improvement in efficiency for hybridoma screening practices with the introduction of FlexAble Antibody Labeling Kits.

Technology

FlexAble Antibody Labeling Kits provide a quick and easy way to label antibodies with fluorophores, enzymes, and other moieties using a polypeptide "FlexLinker". The FlexLinker binds the Fc portion of the primary antibody in a site-specific manner to ensure labeling does not interfere with the antigen recognition sites. The FlexLinker is not covalently bound to the antibody but demonstrates an extremely high affinity and will not dissociate from the target antibody under normal assay conditions.

Unlike covalent conjugation chemistries, FlexAble kits work in the presence of BSA, glycerol, EDTA, FBS, sodium azide, and other components found in common antibody storage buffers and cell culture media. They can also label small amounts of antibody, circumventing the need for large amounts of concentrated antibody that are required by traditional covalent methods.

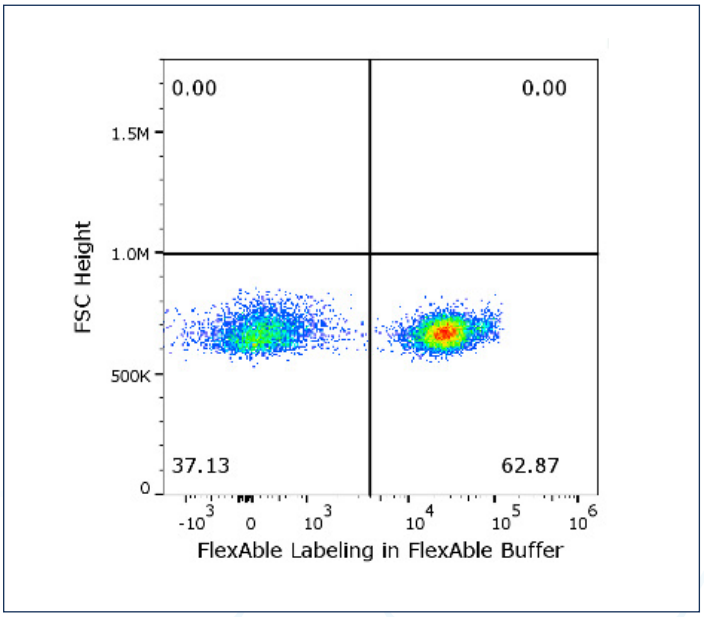
While the main application for FlexAble Antibody Labeling Kits is life science research assays, the kits can also be used to efficiently screen antibody hybridomas. To test this hybridoma screening application, an experiment was devised to test a lower limit threshold for labeling of antibodies present in tissue culture supernatant.

Experiments

Hybridoma supernatant may contain low concentrations of antibodies (10-200 µg/ml), often below the 0.5 µg standard amount referenced in the FlexAble Antibody Labeling Kit protocol. Cell culture supernatant also has a complex composition that had not been previously tested by Proteintech scientists with the FlexAble kits. The following experiments were set up to address both challenges.

Antibody labeling in the presence of tissue culture media

We began by testing an antibody (anti-CD3 Mouse IgG1 antibody, clone UCHT1) with the standard FlexAble protocol and buffer at the lowest recommended antibody amount (0.5 µg) to determine a baseline of labeling and antibody performance. The labeled antibody was used to stain 1x10^6 human peripheral blood mononuclear cells (PBMC) and detected by flow cytometry (Figure 1).



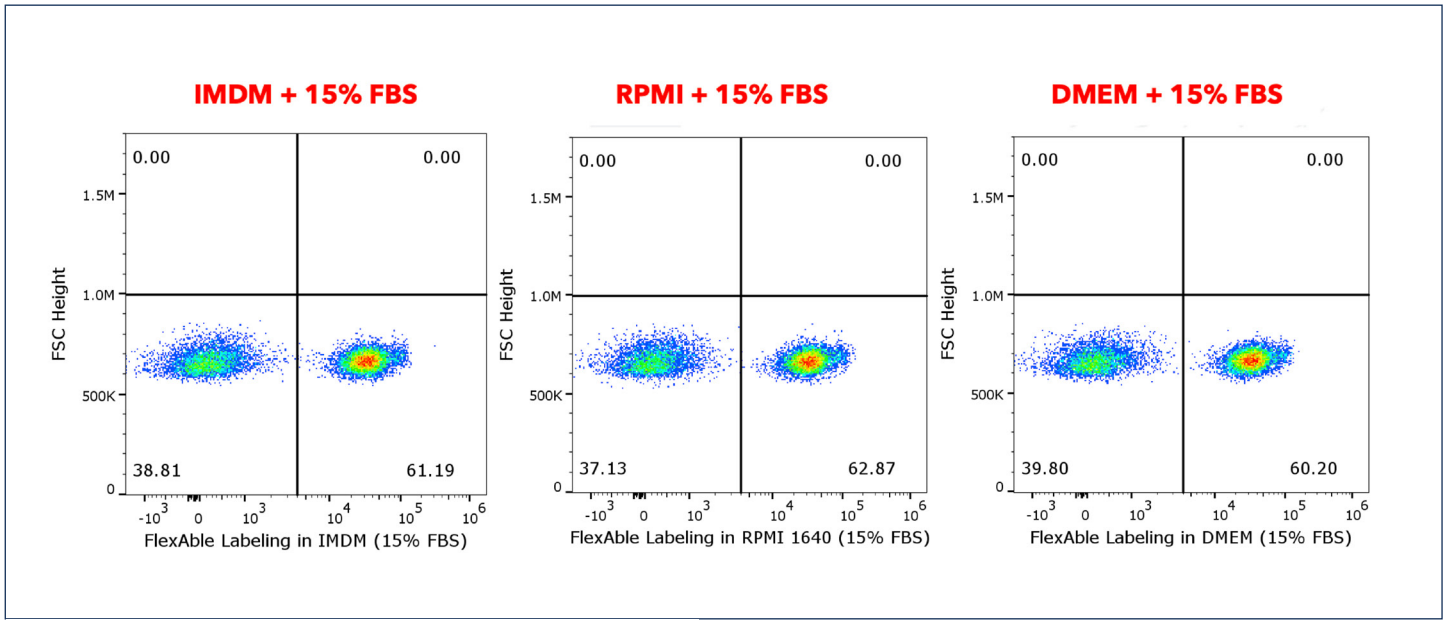
▲ **Figure 1:** Standard FlexAble Buffer. CD3 (UCHT1, Mouse IgG1) was labeled with FlexAble Antibody Labeling Kit for Mouse IgG1 (cat# KFA023).

• 100 µL reaction volume • 1X FlexAble Buffer

• 1 µL FlexAble CoraLite® Plus 647 FlexLinker • 0.5 µg purified antibody

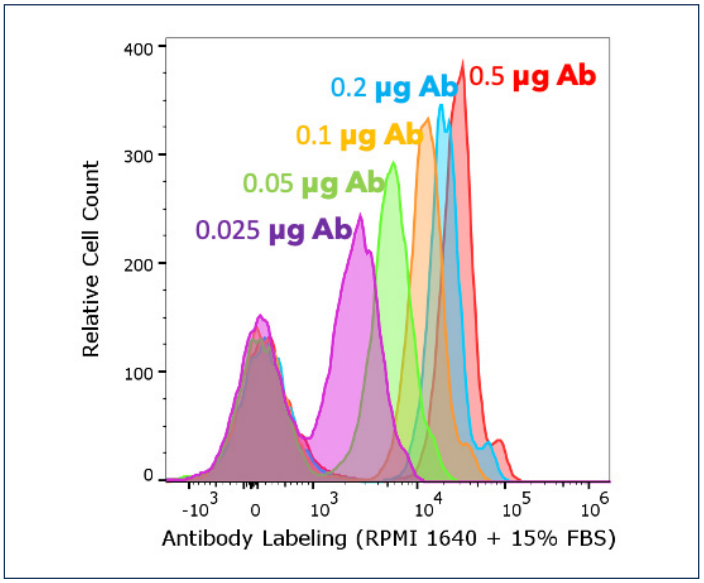
Incubation: 5 min | Staining: 1X10^6 human PBMC cells

The flow cytometry data showed a clean separation of CD3+ and CD3- cells, indicating that the FlexAble labeling of the CD3 antibody and staining of PBMCs with the labeled antibody was successful.



▲ **Figure 2.**

Next, the same purified CD3 antibody was diluted with 3 different tissue culture media mixtures and labeled with the FlexAble CoraLite® Plus 647 Antibody Labeling Kit for Mouse IgG1. The 3 mixtures of labeled CD3 antibodies were used to stain 1x10^6 human PBMCs and detected by flow cytometry (Figure 2). The antibody labeled in the tissue culture medias performed identically to the antibody labeled in the FlexBuffer included in the FlexAble Antibody Labeling Kits, indicating that the antibody labeling done in cell culture media was successful and provided equivalent performance.



Labeling small amounts of antibody

To address the low antibody concentrations found in hybridoma supernatants, a few experiments were set up to establish a baseline readout of known antibody amounts and then test actual hybridoma supernatants against them.

First, the same antibody used in the tissue culture media experiment (anti-CD3, Mouse IgG1 antibody, clone UCHT1) was serially diluted with RPMI 1640 cell culture media with 15% FBS. These samples were labeled with FlexAble CoraLite® Plus 647 Antibody Labeling Kits for Mouse IgG1 and used to stain 1x106 human PBMCs. Relative cell count was measured by flow cytometry (Figure 3).

▲ **Figure 3:** FlexAble Labeling within RPMI 1640 + 15% FBS

CD3 (UCHT1, Mouse IgG1) was labeled with FlexAble CoraLite® Plus 647 Antibody Labeling Kit for Mouse IgG1 (cat# KFA023)

• 100 µL reaction volume • 0.5-0.025 µg purified antibody diluted in

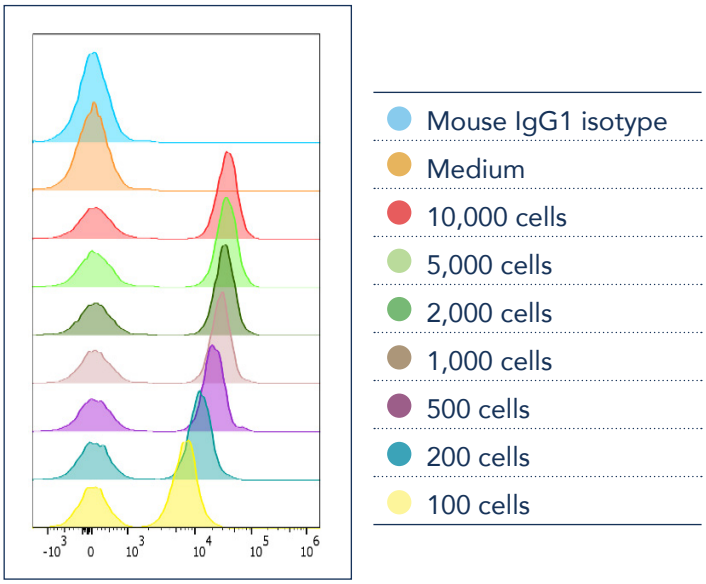
• 1X RPMI 1640 + 15% FBS • 1 µL FlexAble CoraLite® Plus 647 FlexLinker

Incubation: 5 min | Staining: 1X10^6 human PBMC cells

Results show that the FlexAble Antibody Labeling Kits were able to successfully label antibody amounts as low as 0.025 µg in RPMI 1640 with 15% FBS for this UCHT1 antibody.

Testing Hybridoma Supernatant

In a second experiment, CD3 (UCHT1) hybridoma cells were seeded in a 96-well plate at determined amounts and grown for 72 hours at 37 °C. After incubation for 72 hours, 100 µl of supernatant was collected after centrifugation of the plate. 1 µl of FlexLinker was added to the 100 µl of collected hybridoma supernatant and incubated at room temperature for 5 minutes. 1x10^6 human PBMCs were stained with the FlexAble labeled hybridoma antibody and detected using the same flow cytometry protocol used in earlier experiments (Figure 4).



Labeling performed by FlexAble Antibody Labeling Kits was consistent for seeded hybridoma cell amounts between 10,000 cells and 1,000 cells. Decreased signal is observed at 500 cells and below.

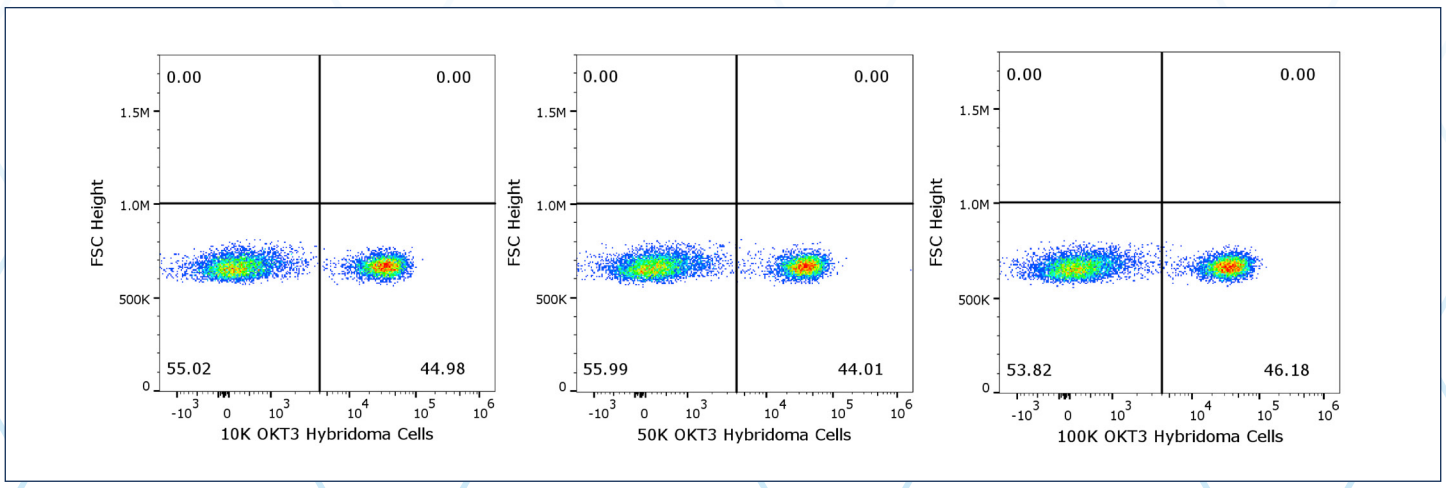
A similar experiment was performed with a different CD3 clone, OKT3. CD3 (Mouse IgG2a, clone OKT3) hybridoma cells were seeded into a 96-well plate at determined amounts and grown for 48 hours. After incubation for 48 hours, 100 µl of supernatant was collected after centrifugation of the plate. 1 µl of CoraLite® Plus 647 FlexLinker for Mouse IgG2a was added to the 100 µl of collected hybridoma supernatant and incubated at room temperature for 5 minutes. 1x10^6 human PBMCs were stained with the FlexAble labeled hybridoma antibody and detected by flow cytometry (Figure 5).

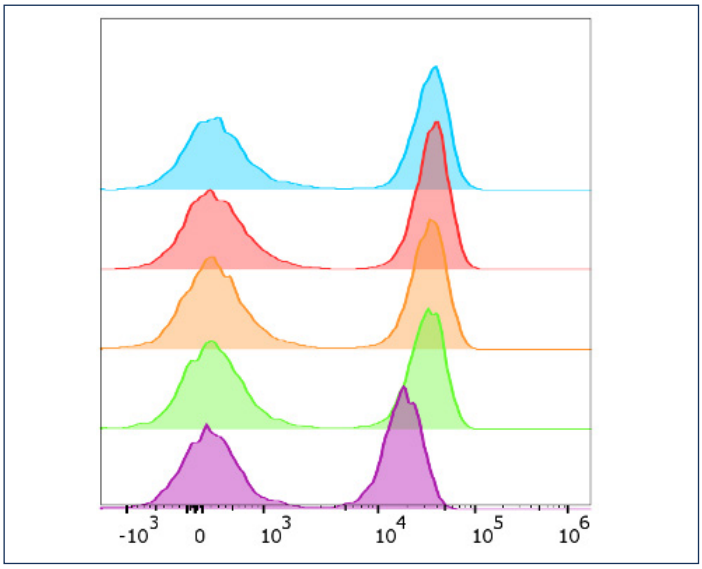
Labeled antibodies in hybridoma supernatants of 10,000, 50,000, and 100,000 seeded CD3 (OKT3) hybridoma cells performed similarly by flow cytometry when applied to 1x10^6 human PBMCs.

A parallel experiment was conducted by labeling known amounts of purified CD3 (OKT3) Mouse IgG2a antibody serially diluted in 100 µl IMDM and 15% FBS. 0.5 µg of purified CD3 (OKT3) Mouse IgG2a antibody was stained in the FlexBuffer included in the FlexAble Antibody Labeling Kits as a standard reaction control. The labeled antibodies were used to stain 1x10^6 human PBMCs and detected by flow cytometry (Figure 6).

▲ **Figure 4:** Readouts of labeled antibody harvested from the supernatant of CD3 (UCHT1) hybridomas. Cells were seeded into 96-well plates in varying amounts and FlexAble CoraLite® Plus 647 Antibody Labeling Kit for Mouse IgG1 was used to label antibodies in the supernatant. The supernatants containing labeled antibody were used to stain 1x10^6 human PBMCs. Positively stained cells were detected by flow cytometry.

▼ **Figure 5.**





Purified OKT3 (Mouse IgG2a) Antibody
Standard Reaction : Label 0.5 µg antibody, 10 µL volume with FlexBuffer
Label 0.5 µg antibody, within 100 µL IMDM + 15% FBS
Label 0.2 µg antibody, within 100 µL IMDM + 15% FBS
Label 0.1 µg antibody, within 100 µL IMDM + 15% FBS
Label 0.05 µg antibody, within 100 µL IMDM + 15% FBS

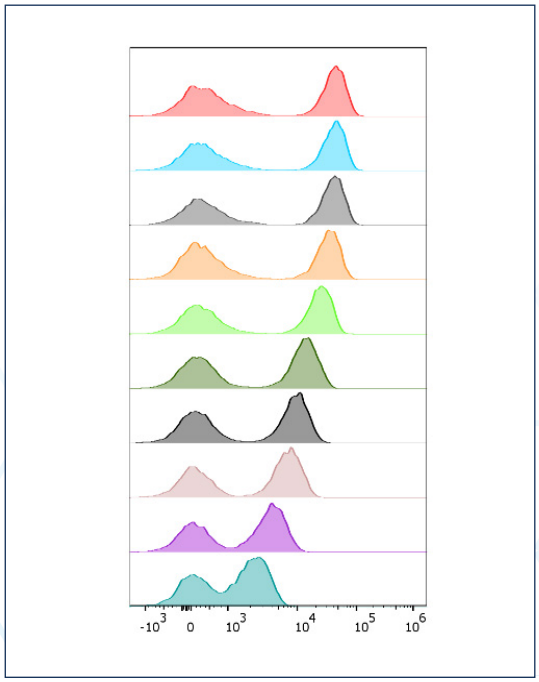
◀ **Figure 6.**

Antibody amounts of 0.5 µg, 0.2 µg, and 0.1 µg performed similarly by flow cytometry analysis. The smallest quantity of antibody, 0.05 µg, showed slightly decreased signal.

Lastly, a serial dilution of hybridoma supernatant was performed and compared to two known amounts of purified antibody to determine the approximate amount of antibody that was labeled. These experiments were performed to test the lower limit of labeling ability for the FlexAble Antibody Labeling Kits with the CD3 (OKT3) antibody clone.

Original hybridoma supernatant from a T-75 size tissue culture flask was collected and serially diluted in IMDM with 15% FBS. 100 µl of supernatant or diluted purified antibody were labeled by adding 1 µl of FlexLinker from the FlexAble CoraLite® Plus 647 Antibody Labeling Kits for Mouse IgG2a and incubating for 5 minutes at room temperature. The labeled antibodies were used to stain $1x10^6$ human PBMCs and detected by flow cytometry. Two results from Figure 6 for 0.2 µg purified antibody and 0.05 µg purified antibody are presented alongside the serial dilutions of hybridoma supernatant (Figure 7).

Starting around the 16-fold dilution, the signal shifts slightly to the left showing decreased staining which corresponds with approximately 0.05 µg of antibody. These results indicate that FlexAble Antibody Labeling Kits are sensitive and robust, allowing for antibody labeling below the standard reference of 0.5 µg of antibody for a single labeling reaction.



Purified OKT3 (Mouse IgG2a) Antibody
Original Culture Supernatant (From regular culture T-75 flask)
Hybridoma Supernatant 2-fold dilution
Purified antibody: 0.2 µg
Hybridoma Supernatant 4-fold dilution
Hybridoma Supernatant 8-fold dilution
Hybridoma Supernatant 16-fold dilution
Purified antibody: 0.05 µg
Hybridoma Supernatant 32-fold dilution
Hybridoma Supernatant 64-fold dilution
Hybridoma Supernatant 128-fold dilution

▲ **Figure 7.**

Conclusion

The experiments outlined in this white paper indicate that FlexAble Antibody Labeling Kits can successfully label minute amounts of antibody in unconventional labeling conditions which may assist scientists in using directly labeled antibodies in more experimental settings.

The kits demonstrated efficient labeling in the presence of 3 different cell culture media mixtures with 15% FBS added, and antibodies harvested from hybridoma cell supernatant. The kits also demonstrated successful labeling of smaller quantities of antibody than previously quoted by the kit protocols.

In the final experiment, antibodies were labeled directly in the hybridoma supernatant without any further purification, concentration, or buffer exchange needed. The labeled antibodies performed successfully in flow cytometry experiments immediately after labeling without any additional processing.

Based on these results, we expect that FlexAble Antibody Labeling Kits can be used to efficiently screen hybridomas to find positive clones or to use labeled antibodies in hybridoma supernatant directly in antibody applications without further handling.

It is important to note that not all antibodies will successfully be labeled in extremely low amounts and not all hybridomas produce the same amount of antibodies. Thus, the results are subject to variation due to the intrinsic differences between primary antibodies, hybridomas, and batch-to-batch variation of both. The method described in this white paper can only serve for screening purposes and is not meant as a protocol for validation or quantification of hybridoma antibodies.

FlexAble Antibody Labeling Kits are available with 7 different fluorophore options, Biotin, and HRP. They are currently available for Rabbit IgG, Mouse IgG1, Mouse IgG2a, Mouse IgG2b, Human IgG and Rat Kappa Light Chain IgG. They have been validated in fluorescent staining of cells (IF/ICC) and tissues (IF/fluorescent IHC), chromogenic IHC, fluorescent Western Blot, chemiluminescent Western Blot, Flow Cytometry, Tyramide Signal Amplification (TSA) multiplex experiments, and with streptavidin conjugated fluorescent dyes (Flow Cytometry/IF). They can theoretically be used in any antibody applications using fluorescent or enzymatic detection with biotin or HRP. This includes ELISA, various multiplex assays, and cyclic IF to name a few.

For more information on the FlexAble Antibody Labeling Kits, please visit

www.ptglab.com/products/flexable-antibody-labeling-kits/