FACS & Furious: Enhancing Discovery by Isolating Antigen-Specific Cells

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Abstract

Antibody-secreting cells (ASCs), traditionally identified by CD138 expression, play a crucial role in antibody discovery. We found that many CD138+ cells also express surface IgG. Functional studies confirmed these dual-positive cells actively secrete IgG and can be enriched using our FACS-based antigen sorting strategy.

Applying this workflow across therapeutic targets consistently increased hit rates and antibody diversity. By leveraging transitional ASCs, which simultaneously express and secrete IgG, this approach enhances discovery efficiency and expands the scope of antibody development.

Background

Plasma cells are the terminally differentiated B cells and they are potent IgG secreters, releasing large quantities of antibodies in the bloodstream to mount an effective immune response. Earlier B cell stages, however, do not secrete antibodies, but display surface-bound immunoglobulin (Ig). The transition from early B cells with membrane-bound Ig expression to plasma cells lacking membrane-bound Ig and actively secreting soluble antibodies occurs through alternative RNA processing mechanisms including alternative splicing and polyadenylation[2,3]. This transition has been shown to be non-instantaneous by prior studies[4]. Consequently, a heterogeneous population of transitional cells exists, characterized by both membrane-bound and secreted Ig, prior to maturation into plasma cells.

CD138+ Cells from Mice

Wild-type mice were immunized with Human Serum Albumin (HSA) using a rapid 3-week immunization protocol, followed by a final boost without adjuvant, a four-day rest period, and harvest of draining lymph nodes. Single cell suspensions were prepared from harvested lymph nodes for analysis.

The lymphocytes were stained with Anti-mouse CD138 Brilliant Violet 421 (BioLegend) and analyzed on a MACSQuant 10 flow cytometer (Miltenyi Biotec). **Figure 1** illustrates CD138 expression versus side scatter (SSC) profiles from HSA-immunized mouse lymph node cells. Consistent profiles for CD138 expression were observed across multiple immunization projects and trials. The scatter plot reveals distinct populations: CD138- cells, a spread of low- to mid-expressing CD138 cells, and a distinct population of high CD138-expressing cells. The increased SSC of CD138+ cells aligns with an expected higher granularity of plasma cells due to their expanded endoplasmic reticulum (ER) used in antibody secretion.

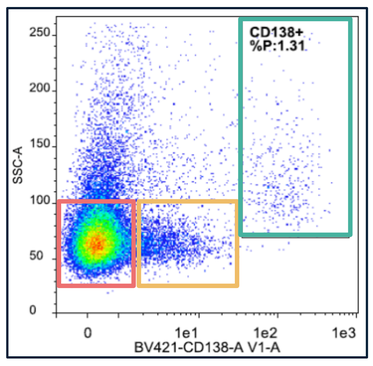

Figure 1. CD138 expression vs. SSC of HSA-immunized mouse lymphocytes, stained with Anti-mouse CD138-Brilliant Violet 421 (BioLegend). CD138-negative cells shown (pink rectangle), with a low-mid shoulder (yellow) of CD138+ cells compared to larger, high-expressing CD138 cells (green).

CD138+/IgG+ Cells Secrete IgG

To investigate the co-expression of CD138 and surface IgG in relation to IgG secretion, lymphocytes from HSA-immunized mice were sorted using the MACSQuant Tyto (Miltenyi Biotec), a microfluidics-based sorter. Cells were gated and sorted into 4 populations: CD138^{lo}, CD138^{mid}, and CD138^{hi}, and CD138^{hi}/HSA+ (the CD138^{hi} population was further enriched for antigen-specific cells by sorting for CD138+/antigen+ subsets). **Figure 2** shows scatter plots with the 3 CD138 sorted cell populations.

Post-sort analysis with a MACSQuant 10 flow cytometer (Miltenyi Biotec) assessed CD138 and IgG surface expression using a secondary antibody to detect IgG (Jackson ImmunoResearch). Antibody secretion was evaluated using the AbTheneum antibody capture workflow, outlined in **Figure 3**. Briefly, sorted cells were seeded into picowells on a microfluidic device, cultured briefly, and then sealed against an antibody capture-functionalized glass slide. Captured antibodies were stained with fluorescently-labeled antigen and labeled secondary antibodies for imaging with a slide scanner (Molecular Devices).

Figure 4 shows the results from assessing the four sorted cell populations. The HSA-positive rate increased with increasing CD138 expression when analyzed by flow. There is a sharp increase in HSA+ cells in the CD138+/HSA+ enriched population (95.8% compared to 37.6% for CD138^{hi}) as expected. A small section of the scanned fluorescent image from the antibody secretion assay is shown after staining with fluorescently-labeled HSA and labeled secondary antibody. The total counts of those hits are overlaid on each image, and the hit rate (ratio of HSA hits over all IgGs) is displayed in the Figure.

The hit rate is increases from 17.6% to 27.4% from CD138^{lo} sample to CD138^{mid}. From CD138^{mid} to CD138^{hi} there is a slight decrease in hit rate (24.5% for CD138^{hi}). It is suspected that the CD138^{mid} population overlaps with the CD138^{hi} and CD138^{lo} samples. If the experiment were repeated, all sort gates could be shifted to bring the CD138^{lo} sample to the low-mid shoulder , similar to the yellow rectangle highlighted in **Figure 1**, to give better separation and phenotypic profiles of the 3 CD138 cell populations.

There is a large increase in hit rate for the enriched CD138^{hi}/HSA+ cell population as expected (64.7%), presenting an opportunity to use this strategy to increase hits in an antibody campaign.

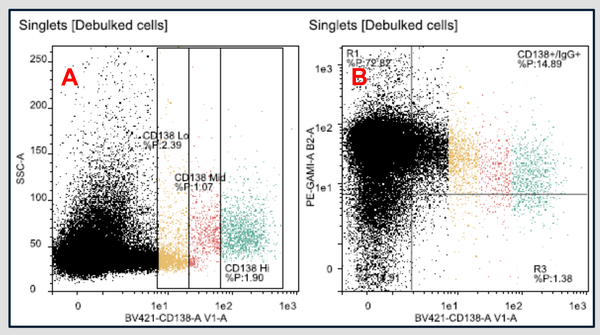

Figure 2. Flow cytometry scatter plots of CD138 sorted mouse cells. **A** shows CD138 expression versus SSC. **B** shows CD138 expression versus IgG expression. Three populations were sorted based on CD138 expression: CD138^{lo} (yellow), CD138^{mid} (pink), and CD138^{hi} (green). The CD138^{hi} population was further enriched for CD138+/antigen+ cells.

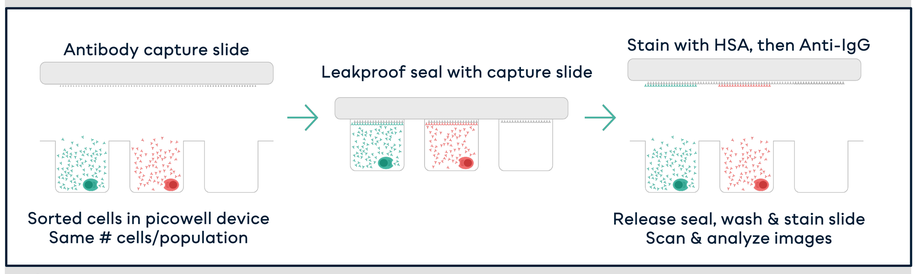

Figure 3. Antibody secretion assay using AbTheneum antibody capture methods. Sorted cell populations from HSA-immunized mice lymphocytes were deposited onto several picowell devices. All secreted IgGs are captured on an antibody capture slide and assessed for antigen-binding by staining with fluorescent HSA. Images from fluorescent scans are analyzed to determine antigen hit rate vs. all IgGs captured.

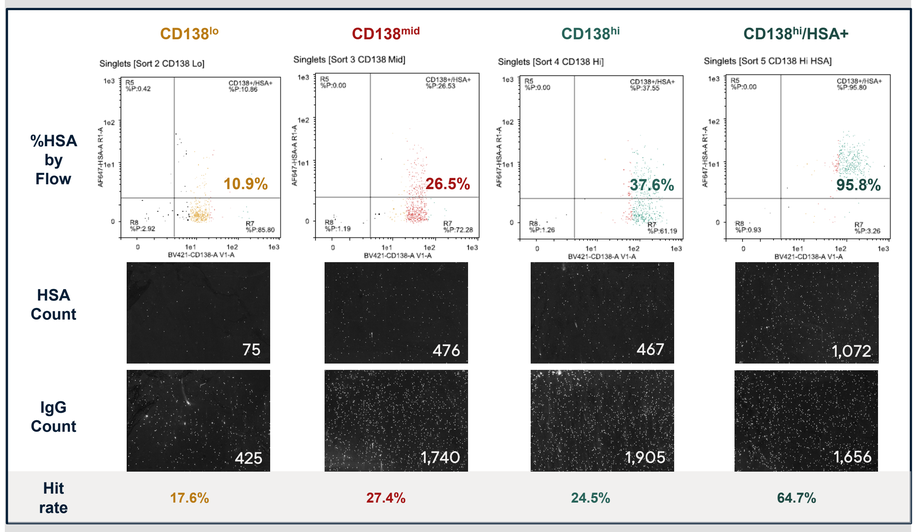

Figure 4. Four sorted cell populations representing a continuum of CD138 expression from HSA-immunized mouse lymphocytes labeled at the top. The % population of HSA-positive hits by flow is displayed in the scatter plots. Cropped images from a small section of the fluorescent scanned image from the antibody secretion assay are shown with the total count of anti-HSA hits and the total count of IgGs secreted from single cells, detected with fluorescently-labeled HSA and a fluorescent secondary antibody, respectively. The hit rate for each cell population is the ratio of HSA hits/all IgGs.

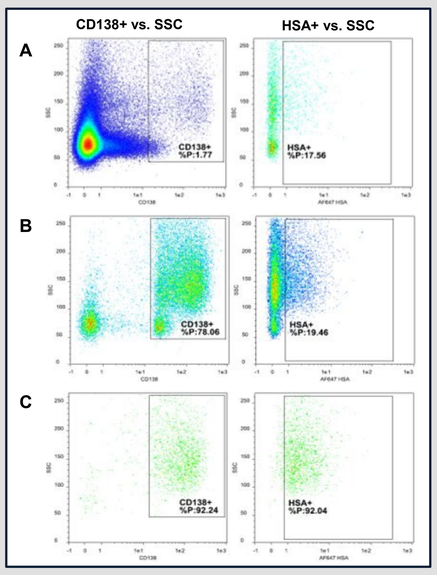

Figure 5. FACS antigen workflow using HSA immunized mouse lymphocytes, **A** shows cell populations before sorting, with the CD138+ staining profile outlined in Figure 1, **B** shows the cells after Sort 1 for high CD138 expressers, with HSA+ cells comprising 19.46%, **C** shows the cell CD138 and HSA expression after Sort 2, which sorts for high CD138+ and positive HSA+. The HSA+ cell population increased from 19% to 92% after Sort 2.

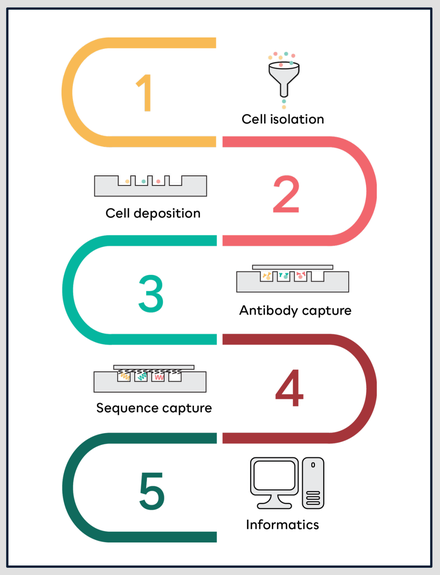

Figure 6. AbTheneum workflow from cells to cross-referenced dataset. (**1**) Sorted cells from the FACS antigen workflow are (**2**) deposited onto a picowell device, (**3**) antibodies are captured and screened. All cells are lysed (**4**) and IgG encoding mRNA is captured and sequenced by NGS. The sequences are mapped back to screened properties using (**5**) informatics. The workflow takes 3-4 weeks.

Table 1. Comparison of hits delivered using magnetic cell isolation for CD138+ cells versus FACS antigen+ workflow (established above with HSA-immunized mice) in 6 different therapeutic antibody campaigns running AbTheneum antibody discovery. The Hits Delivered are the number of antigen-specific antibody sequences delivered in the campaign.

Antibody Campaign	Animal Strain	Cell Isolation	Immunization Duration	Fresh/Frozen	Hits Delivered	% increase in hits
IO Target 1	Transgenic	MACS CD138+	35 Days	Fresh	351	40%
	Transgenic	FACS antigen+	56 Days	Fresh	491	
IO Target 2	Transgenic	MACS CD138+	35 Days	Fresh	456	109%
	Transgenic	FACS antigen+	35 Days	Frozen	954	
IO Target 3	Transgenic	MACS CD138+	49 Days	Fresh	201	50%
	Transgenic	FACS antigen+	63 Days	Frozen	302	
IO Target 4	Transgenic	MACS CD138+	63 Days	Fresh	265	102%
	Transgenic	FACS antigen+	91 Days	Frozen	535	
IO Target 5	Wild-type	MACS CD138+	35 Days	Fresh	456	165%
	Wild-type	FACS antigen+	35 days	Frozen	1,210	
IO Target 6	Transgenic	MACS CD138+	49 Days	Fresh	364	54%
	Transgenic	FACS antigen+	49 days	Fresh	562	

FACS Antigen Enrichment Workflow

Our antigen enrichment workflow aims to enhance antibody discovery by isolating antigen-specific, antibody-secreting cells. Traditional sheath-based sorters dilute sorted populations, which limits their utility for rare cell subsets. The MACSQuant Tyto sorter maintains high cell concentrations, an advantage for identifying rare hits and reducing their losses.

This two-stage sorting strategy begins with lymphocytes stained for CD138 and fluorescently labeled antigen. In the first sort, cells are gated for high CD138 expression (typically ~1% of the population). These sorted cells are immediately transferred into a new sorting cartridge for the second stage, enriching for CD138+/antigen+ cells.

Figure 5 illustrates this workflow and its application. First, cells are sorted for high CD138 expression in Sort 1. The antigen-positive (HSA+) population after Sort 1 is 19%, a slight enrichment from 17% in the pre-sorted cells. The sorted cells from Sort 1 are loaded into a new sorting cartridge and sorted for high CD138- expressing, antigen-positive cells for Sort 2. An increase in the antigen-positive populations from 19% post-Sort 1 to 92% post-Sort 2 demonstrates the dramatic increase capable in this workflow. The sorted cells from Sort 2 are then available to use in AbTheneum antibody discovery workflow, outlined in **Figure 6**. AbTheneum antibody discovery integrates antibody screening with antibody sequencing on a parallel platform from antibody-secreting cells. Commercial kits for isolating CD138+ cells have been used with repeated success, and cells isolated from the FACS antigen enrichment workflow are also compatible.

FACS Antigen Enrichment Always Improves Hit Rate

The FACS Antigen Enrichment Workflow was applied across multiple antibody discovery campaigns targeting immuno-oncology (IO) antigens using AbTheneum antibody discovery. Results from **Table 1** demonstrate the workflow's impact on improving hit rates and antibody discovery efficiency compared to standard magnetic CD138+ cell isolation.

Traditional MACS-based CD138^{+} enrichment yielded a baseline population of antibody-secreting cells but lacked specificity for antigen-positive cells, which can limit hit rates and diversity. In contrast, the FACS-based approach enriched for CD138^{+}/antigen^{+} cells, leveraging transitional ASCs with dual IgG secretion and surface IgG expression. This targeted method consistently improved performance, delivering 50%–165% more hits across campaigns. Notably, for IO Target 5 in wild-type animals, hits increased from 456 with MACS CD138^{+} enrichment to 1,210 with FACS antigen^{+} sorting, representing a 165% improvement.

The workflow also demonstrated robustness when applied to challenging conditions, including extended immunization durations and the use of frozen lymphocytes. For IO Target 4, extending the immunization from 63 to 91 days and utilizing frozen cells doubled the number of hits, increasing from 265 to 535. Similarly, for IO Target 3, extending the immunization period from 49 to 63 days and transitioning to frozen cells improved the hit rate by 50%, from 201 to 302. These findings highlight the workflow's ability to enhance discovery outcomes even under conditions that traditionally reduce cell yield or viability.

The FACS Antigen Enrichment Workflow proved effective across both transgenic and wild-type mice, with similar benefits observed in both genetic backgrounds. Additionally, while fresh cells generally performed better, frozen cells sorted using this workflow delivered competitive results. For IO Target 2, frozen cells yielded 954 hits, a 109% improvement over fresh cells enriched by MACS CD138^{+} sorting.

Overall, the FACS Antigen Enrichment Workflow not only improved hit rates but also broadened antibody diversity, confirmed by the sequence data from the hits which deliver overlapping clonotypes and unrepresented clonotypes in the MACS CD138+ sorted sequence dataset (data not shown). Specifically, the workflow consistently yielded a 50-165% increase in hits and demonstrated effectiveness across diverse targets, immunization protocols, and sample conditions. These findings demonstrate the workflow's scalability, reproducibility, and utility in diverse antibody discovery campaigns, further supporting its integration into modern antibody discovery pipelines.

Conclusions

Our study highlights two significant findings. First, CD138+ cells demonstrate dual functionality by expressing surface IgG and actively secreting it. This discovery underscores the biological importance of transitional ASCs in antibody research and provides new insights into their utility in antibody discovery workflows.

Second, we demonstrate a strategy for enriching antigen-specific, antibody-secreting cells to increase hit rates in an antibody discovery campaign. The FACS Antigen Enrichment Workflow offers a robust and reproducible strategy for enriching antibody-secreting cells with a recombinant protein target. This workflow consistently enhances hit rates and antibody diversity across various discovery campaigns. It is versatile and compatible with diverse immunization protocols, protein targets, and sample conditions, making it a valuable tool when integrated with AbTheneum antibody discovery. These findings illustrate the dual benefit of advancing our biological understanding of transitional ASCs while optimizing workflows to support therapeutic antibody discovery.

Contact

Learn more about AbTheneum antibody discovery at www.singlecelltechnology.com
Or scan the QR code here

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