Webinar Summary

Next-generation biomanufacturing: Process intensification for monoclonal antibody production

Though monoclonal antibodies (mAbs) are comparatively well-characterized and often commercialized in contrast to many other biotherapeutics, there still exists significant opportunity to intensify upstream manufacturing processes to improve efficiency and promote sustainability. Upstream intensification can be accomplished in several ways, such as by employing high cell density or high-volume cell banks during the seed train step, or by employing concentrated fed-batch or continuous perfusion processes. Additionally, there are several downstream process improvements that can enhance efficiency and improve quality, including the use of high-affinity resins, next-generation polishing strategies, and innovative chromatography systems.

Regardless of how operators choose to pursue process intensification, the right cell culture media, bioreactor systems, chromatography systems, and selection of consumables are fundamental to success. To promote process intensification for mAb applications, Thermo Fisher Scientific has worked to optimize its Gibco™ Cell Culture Media, applying proteomics and metabolomics to inform more targeted media formulations. Moreover, these formulations are available in Thermo Fisher's advanced granulation technology (AGT) format, which allows for faster dissolution, shorter mixing times, and pre-calibrated auto pH.

Leveraging these next-generation media technologies can help streamline and simplify the processes, achieve higher titers, and optimize scale-up. This, coupled with optimized and intensified downstream process strategies, can enable biomanufacturers to increase productivity, simplify workflows, and reduce overall costs for mAb production.

Advancing mAb bioprocessing: upstream innovations

In cell line development (CLD), drug developers are seeking high titers, short timelines, a host cell line that is regulatorily compliant, and low licensing costs. At Thermo Fisher Scientific, we offer strategic implementations to maximize cell growth and productivity while streamlining your processes. From there, we can identify suppliers that can deliver the starting materials and technologies to help your workflow thrive.

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Key to upstream intensification is the development and use of a suitable chemically defined media and feeds to support CHO-based fed-batch and/or perfusion processes. For fed-batch processes, the Gibco™ Efficient-Pro™ Medium and Feed System consists of a single medium applicable across all CHO cell variants, which can be paired with two cell line-specific feeds respectively:

- Efficient-Pro Feed 1, designed for CHO-K1 and CHO-K1 GS cell lines
- Efficient-Pro Feed 2, designed for CHO-S, DG-44 and CHO-K1 GS cell lines
- Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer designed for CHO-K1 GS cell line

Leveraging omics-driven design (namely metabolomics and proteomics analysis), the Efficient-Pro Medium and feeds can enhance specific productivity, enabling higher antibody yields and improved process efficiency. For perfusion-based applications, the Gibco™ High-Intensity Perfusion (HIP) CHO Medium has been developed to meet the altered nutritional demands of continuous culture. As a chemically defined medium, HIP-CHO supports multiple CHO lineages and enables high cell densities and productivity at relatively low perfusion rates. A key advantage of HIP-CHO is its flexibility in rehydration concentrations, allowing adaptation to various perfusion strategies, including (but not exclusive to) N-1 perfusion and continuous perfusion processes. This adaptability enhances process consistency and scalability while maintaining optimal cell health and productivity.

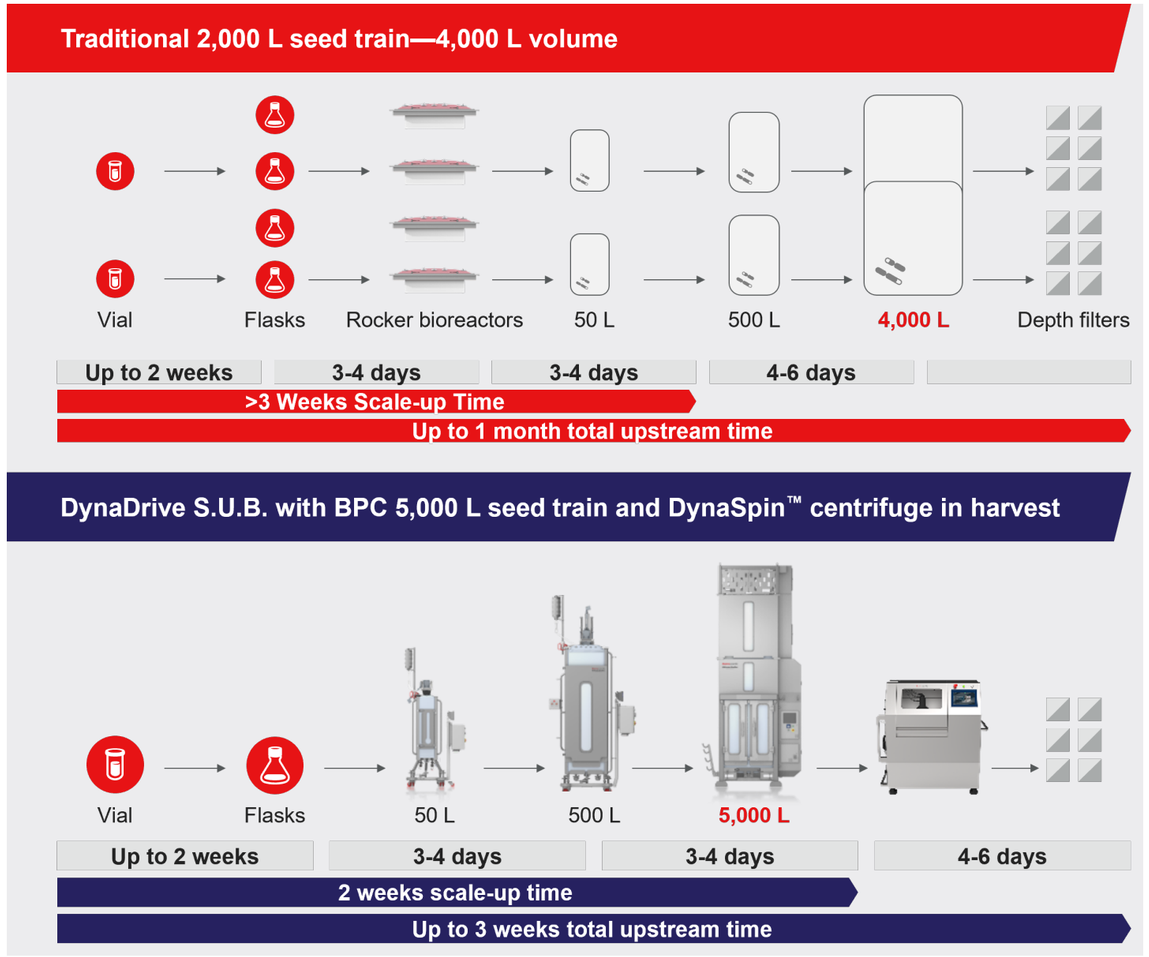
Bioreactor innovation likewise plays a crucial role in upstream process intensification. Thermo Fisher's latest generation of high-performance single-use bioreactors, the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), offers superior power mixing, mass transfer, and scalability. These bioreactors feature high turndown ratios — from 10:1 to 20:1, depending on scale — enabling seamless N-1 seed train steps within the same vessel. Available in capacities from 5 L to 5,000 L, the DynaDrive bioreactor reduces the number of seed train steps and shortens the overall upstream process timeline.

By combining high turndown ratios and intensified fed-batch processing, the DynaDrive S.U.B. enables a reduction in total process time by approximately one week. This results in an estimated cost savings of over 30% in both capital expenditures and operational expenditures, making it a highly efficient solution for commercial scale mAb production.

In a recent case study, Thermo Fisher demonstrated the scalability of a CHO-K1-based continuous perfusion process utilizing HIP-CHO Medium in a high-performance bioreactor setup. The process was initially developed in a 3 L bioreactor and successfully scaled to a 50 L DynaDrive bioreactor. Key findings from the study include:

- CHO-K1 cells with HIP CHO Medium in continuous perfusion showed similar high cell growth and viability with scale-up from the Thermo ScientificTM HyPerforma™ glass bioreactor to the 50 L DynaDrive S.U.B.
- With a low perfusion rate of 1 vessel volume per day, the medium supported a peak viable cell density (VCD) of ≥110 x 106 cells/mL and an average of 83 to 93 x 106 cells/mL during steady state on days 10 through 50 (VCD SS avg).
- With scale-up to 50 liters, high cell viabilities of >90% were maintained during steady state with average viabilities of 94% to 96%.
- During scale-up, similar and consistently high bioreactor titers ≥1.5 g/L/day with averages of 1.7 to 1.9 g/L/day during steady state were observed from days 10 through 50.
- Across scaling, the medium supported similar cell-specific productivities with steady-state averages of 13 pg/cell/day in the Hyperforma glass bioreactor and 14 pg/cell/day and 17 pg/cell/day, respectively, in two separate runs in the 50 L DynaDrive S.U.B.
- Using an average 20% bleed rate and resulting 80% effective harvest rate, HIP CHO Medium supported an equivalent average of 1.4 g/L/day volumetric harvest productivity across the 3-liter and two 50-liter runs during steady state.
- CHO-K1 cells with HIP CHO Medium produced consistently strong total harvest yields of 57 g/L in the Hyperforma glass bioreactor and 55 g/L and 60 g/L, respectively, for each run in the 50 L DynaDrive S.U.B.
- The total process harvest yields of 2,700 g and 3,000 g of mAb product demonstrate the high efficiency of a continuous perfusion process with modest scale-up to 50 L.

Note:
The Hyperforma glass bioreactor run experienced issues at Day 30 with a defective pH probe and required external pH measurement through the remainder of the run. The first 50-liter run experienced an equipment connection issue late in the run that contributed to the drop in bioreactor titers from day 46 through day 50.



An illustration of how the DynaDrive bioreactor can accelerate scale-up.

Eliminating traditional inoculum expansion for faster bioprocessing

One of the most effective ways operators can streamline operations is by eliminating traditional inoculum production systems, such as shake flasks, wave-mixed bioreactors, or stirred bioreactors, and directly inoculating a pilot bioreactor from a single cryovial. In a case study, researchers from ZHAW Zurich University of Applied Sciences bypassed the need for separate inoculum production steps and instead directly transferred cryopreserved cells from a high-density cell bank to a 50 L pilot bioreactor. Using an ultra-high-density cell bank with a cell density of 260 million cells per mL and a DynaDrive S.U.B. 50 L high-performance bioreactor with a turndown ratio of 1:10, a fed-batch process was initiated without requiring separate inoculum expansion steps.

To initiate the study, a single cryovial (4.5 mL) of ultra-high density cell suspension of Gibco™ ExpiCHO-S™ mAb producing cell line was thawed and directly transferred into the 50 L DynaDrive S.U.B. with an initial volume of 5 L. Over six days, the working volume was increased from 5 L to 38 L using a chemically defined Efficient-Pro Medium. On Day 6, daily feeding with Efficient-Pro Feed 2 was initiated to promote mAb production. For comparison, a standard shake flask-based inoculum expansion system was used as a reference.

A comparative analysis between the 50-liter DynaDrive S.U.B. and a conventional shake flask process revealed similar cell growth kinetics, with both systems reaching comparable relative cell densities. Despite differences in absolute cell density due to the dilution effect in the bioreactor, the overall trends in growth remained consistent. Similarly, the IgG titer profile followed an almost identical trajectory between the two systems, yielding antibody concentrations of 3.7 grams per liter in the bioreactor, comparable to the 3.6 grams per liter obtained in the reference system. Further quality assessments demonstrated similar charge variant distributions and glycosylation patterns, with high monomeric content exceeding 90% across all cultivation conditions.

By eliminating traditional inoculum production steps, this approach reduced the inoculum production phase from ten days to just four. The results confirmed that not only were growth and productivity outcomes equivalent to conventional methods, but critical quality attributes of the final product were also preserved.

Ultimately, researchers were able to demonstrate that direct bioreactor inoculation using an ultra-high-density cell bank is a viable strategy for improving efficiency in monoclonal antibody production. By reducing equipment footprint, minimizing process complexity, and accelerating timelines, this method can offer significant advantages for upstream bioprocess intensification.

The impact of high-density cell banking on seed train efficiency

The adoption of high-density cell banking represents a transformative shift in mAb seed train intensification. Traditional workflows involve scaling up from a 1 mL cryovial through multiple shake flask stages into rocker bags before reaching the production bioreactor. In contrast, utilizing high-volume, high-density cell banks within bioprocessing containers enables direct inoculation of pilot-scale and production-scale bioreactors, such as the 500 L and 5,000 L DynaDrive S.U.B., thereby streamlining the process.

To implement this approach, cell banks are generated using perfusion technology. High-intensity perfusion cultures with high-producing cell lines are scaled up in traditional shake flasks before being inoculated into a 50 L DynaDrive S.U.B. Once optimal densities are achieved, cells are transferred into bioprocessing containers for cryopreservation. This method significantly reduces the duration of the seed train, leading to improved efficiency and cost-effectiveness.

Implementing high-density cell banking offers significant process efficiencies. In a standard seed train, the process may take up to 21 days to reach the production bioreactor. However, by leveraging high-density banks, this duration can be reduced to just six days. The labor requirements are also significantly decreased, reducing hands-on operating time from 66 hours to 37 hours. Additionally, workforce demands can be minimized, cutting the number of personnel required from approximately 14 in traditional processes to just eight.

By eliminating shake flasks, rocker bags, and intermediate seed train steps, the use of high-density cell banks also results in substantial savings in consumables. In one model, consumables costs were reduced from $100,000 to $90,000 (USD). The reduction of unit operations contributes to a smaller facility footprint, freeing up space for other critical operations and enabling more efficient use of bioprocessing suites.

The high turndown ratio of DynaDrive S.U.B. facilitates direct inoculation of the N-2 bioreactor, allowing rapid culture expansion before transfer to production-scale bioreactors. This enables faster turnaround times between batches, increasing equipment utilization. Compared to traditional processes, where the N-1 bioreactor is occupied for extended periods, the intensified workflow allows for more frequent batch production. This efficiency can translate to an additional batch per year in some cases.

Validation studies have confirmed that high-density banks maintain cell viability and productivity. Growth curve analyses show that post-thaw recovery and expansion rates are consistent across different bank densities, with no significant deviations in doubling time, viability, or growth kinetics. Furthermore, perfusion-derived banks demonstrate comparable performance in fed-batch cultures, achieving similar peak cell densities and antibody titers to traditional seed trains.

Downstream intensification: flow-through vs. bind/elute

The downstream purification of mAbs is a critical step in bioprocessing, requiring solutions that enhance efficiency while maintaining high product quality. As the industry faces increasing pressure to reduce costs and improve process throughput, the implementation of high-capacity, high-throughput, and high-resolution purification technologies has become essential, as these advancements not only contribute to lower cost of goods but also streamline processing workflows. High-resolution resins facilitate the separation of closely related product variants, simplifying purification steps and improving overall yield.

Thermo Fisher Scientific offers a broad portfolio of high-performance purification solutions, including affinity, ion-exchange, hydrophobic interaction and mixed-mode chromatography resins. The affinity purification step is central to downstream intensification, as it dictates overall process efficiency. Its CaptureSelect™ technology utilizes unique affinity ligands to enable purification of complex target biomolecules, including monoclonal antibodies, viral vectors, and vaccines.

Furthermore, the latest addition of protein A resin to the affinity portfolio, MabCaptureC™, incorporates an engineered ligand with high binding affinity for the CH2-CH3 interface of human IgG. With an estimated binding capacity of 50 g/L and exceptional alkaline stability, this resin supports over 100 purification cycles with 0.2 M sodium hydroxide, facilitating extended reusability and reduced resin costs. The highly cross-linked agarose backbone of MabCaptureC, featuring uniform bead size at approximately 75 microns, further enhances performance by offering superior scalability and robustness for commercial manufacturing applications.

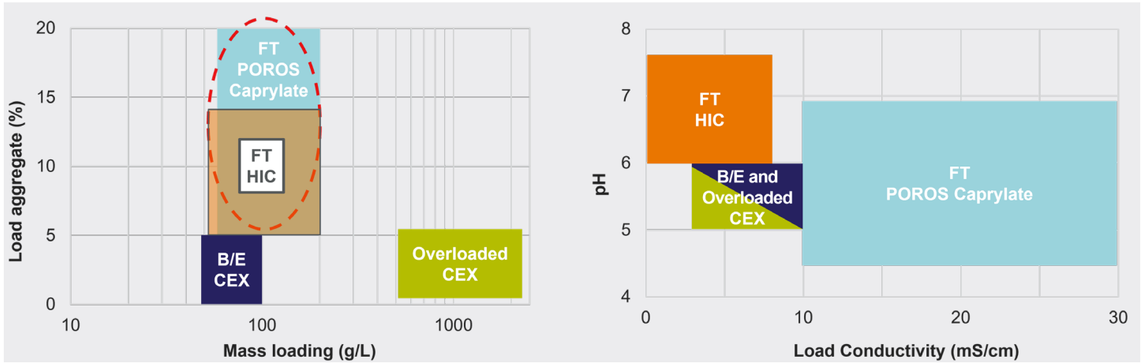
Enhancing the affinity step for downstream intensification generally involves two main strategies: increasing binding capacity and improving reusability. By enabling higher binding capacities, less resin is required, directly impacting process economics. Comparisons between MabCaptureC and competitor resins demonstrate either equivalent or superior binding capacities, both in polyclonal IgG and monoclonal antibody feedstocks, even at varying residence times. Beyond binding efficiency, long-term reusability is critical to maximizing cost savings. Reusability studies conducted with MabCaptureC demonstrate sustained performance across 200 caustic cycles, maintaining consistent yields, binding capacity, and minimal protein A ligand leakage. These findings underscore the resin's resilience against rigorous cleaning procedures, supporting long-term process efficiency.

An emerging trend in DSP process intensification is continuous chromatography. In continuous chromatography, multiple columns or a single column with a continuous feed system are used to maintain a steady state of operation. The process is designed so that while one part of the column is being loaded, another part is being washed, and yet another part is being eluted. This enables a constant flow of purified product without interruption. High-capacity resins like MabCaptureC can be used to further help in improving productivity of the workflow.

While the affinity step forms the backbone of antibody purification, polishing steps are equally important for optimizing product purity. The polishing stage typically involves removing aggregates and other impurities, a process that can be intensified using flow-through chromatography. Compared to traditional bind-and-elute chromatography, flow-through operation offers several advantages, including higher mass loadings, reduced buffer and resin requirements, shorter processing times, and a smaller equipment footprint. Flow-through mode does not facilitate the separation of closely related species, but it does provide a significant advantage in reducing aggregate levels while maintaining high recovery rates.

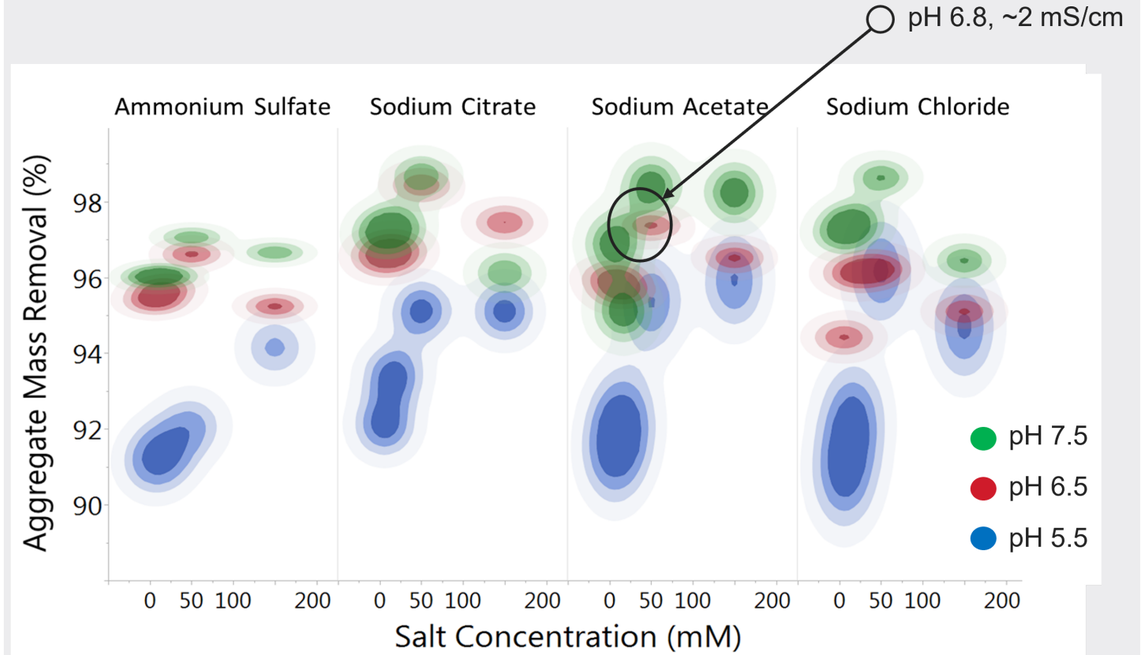
Operating in flow-through mode can significantly help reduce costs associated with resin volume and buffer consumption. To illustrate these cost savings, consider a calculation scenario involving a 200 L bioreactor with a mAb titer of 1g/L. In a conventional cation–exchange bind-and-elute setup, with a resin loading density of 50g mAb/L resin, a 4 L resin volume would be required. In contrast, a flow-through approach can accommodate increased loading densities, often exceeding 200g mAb/L resin, resulting in a 4-fold reduction in resin volume. Additionally, flow-through mode dramatically decreases buffer consumption by eliminating the need for elution buffers and reducing the volumes of wash buffers required. This can lead to an overall 7-fold decrease in buffer usage. The combined advantages of reducing resin and buffer consumption can translate into substantial cost savings and improvements in process efficiency.

The selection of polishing resins for aggregate removal depends on multiple factors, including mass loading capacity and process flexibility. Technologies such as mixed-mode resins and hydrophobic interaction resins (HIC) are well suited for flow-through operation. In particular, POROS™ HIC resins exhibit high mass loading capacities while effectively reducing aggregate levels. These resins also provide a broad operating range, allowing for process flexibility across different conductivity and pH conditions.



Typical operating range for intermediate polishing steps for aggregate removal.

Conditions: 0 - 150 mM Salt, pH 5.5 - 7.5



Initial screening results showing aggregate removal for POROS Benzyl Ultra in flow-through mode. Highlighted condition is the same mobile phase as the previous mixed-mode step.

POROS resins offer several distinguishing features that contribute to their superior performance in chromatography applications. Their rigid poly(styrene-divinylbenzene) backbone facilitates stable column bed integrity and linear pressure-flow performance, enabling high operating flow rates with minimal pressure drop. With an average particle size of 50 microns, POROS resins strike an optimal balance between resolution, productivity, and throughput. Additionally, their large pore structure reduces diffusive mass transfer resistance, allowing for high-capacity separations with minimal performance loss at increased flow rates.

Case studies further illustrate the benefits of using POROS hydrophobic resins in flow-through mode for aggregate removal. One study focused on optimizing the purification of a clinical-stage monoclonal antibody (mAb A) that exhibited high aggregate levels exceeding 12% after initial ion-exchange purification. The initial proposed step for aggregate removal was a mixed-mode resin which achieved 99% purity with 90% recovery at a loading density of 25 g/L resin. By switching to POROS™ hydrophobic resin in flow-through mode, researchers identified optimal buffer conditions that allowed for >90% aggregate removal. Furthermore, they observed good aggregate removal using the same mobile phase conditions as the previous mixed-mode resin. This simplified the DSP as the replacement of the mixed mode by the HIC resin would not undergo additional buffer exchange between chromatography steps. Subsequent experiments confirmed that POROS resins maintained high performance at increased flow rates. At an 800 cm/hr linear velocity and 45-second residence time, the resin effectively removed aggregates while sustaining high monomer recovery, demonstrating its suitability for high-productivity downstream workflows.

In addition to HIC resins, the POROS portfolio now includes its first mixed-mode cation-exchange resin, POROS Caprylate™. Designed for flow-through operation, this resin combines hydrophobic and weak cation-exchange functionalities, enabling efficient aggregate removal and process intensification. Comparative studies between POROS Caprylate and competitor mixed-mode resins show superior performance, with higher monomer recovery rates and improved host cell protein reduction at similar loading densities. The resin's broad operational range across various pH and conductivity levels further enhances process flexibility, making it an optimal choice for streamlined downstream processing.

In summary, the implementation of high-performance resins in both the affinity and polishing steps can effectively achieve downstream process intensification. The combination of high-capacity protein A resins, flow-through polishing strategies, and robust POROS chromatography solutions enables manufacturers to increase productivity, simplify workflows, and reduce overall costs, ultimately driving efficiency in mAb purification.

Interested in leveraging Thermo Fisher Scientific's product lines for mAb development?
Learn more at: **thermofisher.com/mabs**