A New Approach Uses Single Plasma Cells to Generate Higher-Quality Rabbit Monoclonal Antibodies

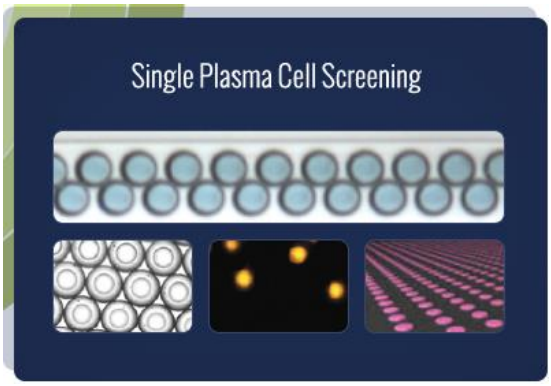
From drug discovery or diagnostic assays to basic research, monoclonal antibodies are common and essential reagents for a wide variety of clinical and research applications. Unfortunately, generating a high-quality antibody is not nearly as straightforward as their popularity might suggest, and reproducibility has been a growing concern (Prassas & Diamandis, 2014; Baker, 2015; Bradbury, A, & Plückthun, 2015).

Over the years, scientists have designed many methods for developing and producing antibodies. Most approaches are still in use today, and they result in a broad range of antibody affinity and specificity. But at this time, there is no easy way to test the effectiveness of an antibody before it is deployed for a project — so researchers often do not know whether they have a high-quality antibody until a costly experiment ultimately fails and they must start the lengthy process all over.

Obviously, guesswork and luck are not hallmarks of repeatable science. A new approach to antibody development aims to eliminate low-quality antibodies from the start so that only those most likely to be successful are moved into a screening pipeline. The method relies on single-cell technology to target only plasma cells, which by nature contain antibodies that are the most mature and are therefore more likely to be effective than the early-stage antibodies scooped up by other techniques. This new approach also expands monoclonal antibody production from the traditional mouse model to rabbits, which can generate more diverse antibodies with higher affinity and greater stability.

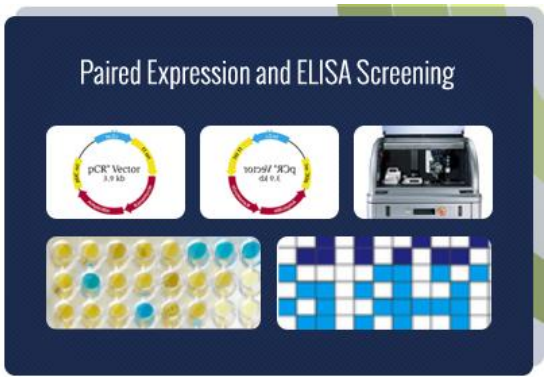
Traditional Antibody Development

Some widely used methods for generating monoclonal antibodies have been around for the better part of a century, while others have been developed in the past few years.



Hybridomas, for instance, are created by immunizing an animal (typically a mouse) with the target antigen and then fusing its spleen cells with myeloma cells. Long considered

the bedrock of antibody development, the hybridoma approach is reliable. However, because it allows any B cell to fuse with myeloma cells, it necessarily includes a high number of naïve or early-stage B cells that are unlikely to produce a high-quality antibody. This forces scientists to screen excessive numbers of hybridoma cell lines — a costly process — to find a cell line that produces an effective antibody for the target antigen. This approach also suffers from very long turnaround times; it can take three to four months to generate the desired antibodies, which slows research in general but is especially problematic for scientists building new, sometimes urgently needed, diagnostic tests.



Another approach that's similar in concept to the hybridoma method is B cell immortalization, which uses the Epstein-Barr virus instead of myeloma cell fusion. The resulting immortalized B cells are excellent for continuously secreting antibodies, but they have the same low efficiency for producing high-quality antibodies as hybridomas. This method is generally considered time-consuming and complicated.

Some of the most commonly used techniques for antibody production are phage display and yeast display. Their major advantage is that they do not require immunization of animals, relying instead on synthesized V-gene libraries. Using the most diverse libraries offers the highest chance of producing an effective antibody, but screening libraries of that size is often prohibitively expensive. When maturation steps are eliminated to keep costs in check, the resulting antibodies tend to have very low affinity.

More recently, scientists have used single-cell techniques to develop antibodies. In one application, individual B cells can be loaded on microtiter plates and cultured until they produce enough antibody for screening. While this method has distinct advantages in turnaround time and streamlined workflow, it can be affected by immature cells as well as growth bias during incubation. To offset growth bias, scientists must use very high numbers of single cells, each plated into a large number of wells in the microtiter plates, restricting the method to well-funded, high-throughput settings for applications such as therapeutic antibody development. A slightly different approach uses single memory cells, which typically contain mature antibodies so there is a higher chance of generating a high-affinity antibody with less screening needed. However, a limiting factor is that memory cell surface markers are not available in all organisms used for antibody production.

Plasma Cells

A new approach to antibody development follows on these single-cell innovations and focuses on

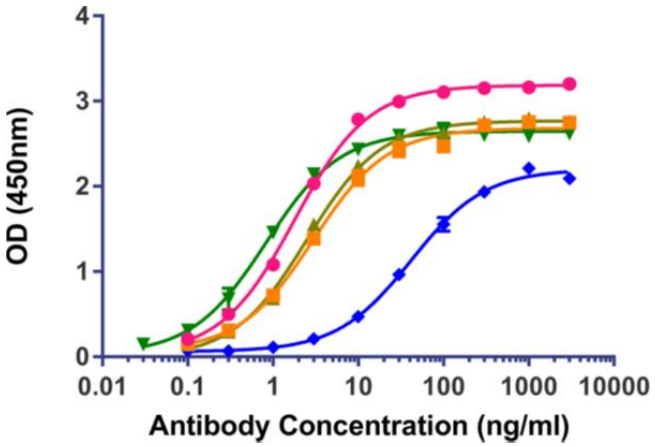


Figure 1. Rabbit anti-Cyclophosphamide monoclonal antibody generated by SPIN tech.

plasma cells, which have orders of magnitude more antibody mRNA than their precursor B cells and contain only mature antibodies. By isolating mature terminal plasma cells, this method makes it far more likely that antibodies generated will have high affinity and specificity. These cells represent as little as 0.1% of the B lymphocytes from spleen in an immunized animal, so there are significantly fewer cells to screen downstream than methods that begin with all B cells. This reduces costs and also shortens antibody development time to only two weeks, a major improvement compared to the months-long turnaround time of other approaches.

This technique is used with rabbits, which have been shown to produce higher-quality antibodies than mice due to their large antibody repertoire. In addition to generating higher-affinity antibodies, rabbits also have more diverse epitope recognition so they can produce a broader range of antibodies than other animals.

By combining these advantages — single cells for an efficient workflow, plasma cells for mature antibodies, and rabbits for more diverse and higher-affinity antibodies — this new approach offers scientists a more effective, reliable means of getting high-quality antibodies quickly.

Table: Anti-Cyclophosphamide clones generated by SPIN tech

Lymphoid Tissue Types	Splenocytes Screened	Antigen-specific Plasma Cells Isolated	HC & LC Pairs Cloned	Indirect ELISA Positive
Spleen	2x10^6	94	65	61

Looking Ahead

Ultimately, producing higher-quality antibodies faster will help eliminate wasted time and resources in basic science and clinical diagnostics development. From diagnostic developers who must wait months between iterations of a new immunoassay to bench scientists who must allocate precious grant funds to screening for the ideal antibody, there is a great deal of opportunity to accelerate clinical research and basic science by starting with more effective antibodies that can be generated reproducibly and quickly.

Tight budgets are a shared theme in academic and clinical settings. If we are to keep making progress, it is imperative that we find ways to accomplish more with each dollar. Adopting approaches that reliably deliver high-affinity, highly specific antibodies is one simple way to reduce experimental costs and accomplish more projects while maximizing chances for scientific success.

References

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