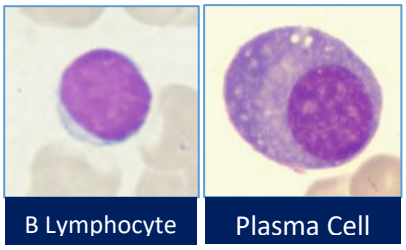
Single plasma cell interrogation SPIN® technology for Monoclonal Antibody Development

The vertebrate immune system, which can recognize a nearly infinite number of possible invaders, is one of the most complicated biological processes. The humoral immune system involves B cells, which normally begin as stem cells in the bone marrow (in mammals), or the bursa of Fabricius (in birds), and then migrate to the spleen. B lymphocytes go through many transitional stages in the spleen, with plasma cells as the final, antibody producing cells, with the highest affinity to antigens. Only 1% of B-lymphocytes are fully developed plasma cells, and it is only the plasma cells that secrete large quantities of antibodies.



B Lymphocyte | Plasma Cell

Obviously, isolation of antigen-specific plasma cells is the most efficient way to isolate high-affinity antibodies. Starting with plasma cells would greatly reduce screening time, and result in antibodies with the highest antigen affinity. Our patented SPIN® technology is the only technique that begins with single plasma cells.

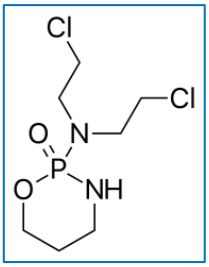
Large scale yet efficient Screening

SPIN® Tech screening:

- > 1,000,000 Splenocytes
- > 100,000 B Lymphocytes
- > 2,000 Plasma cells
- ~ 100 antigen-specific plasma cells

Monoclonal antibody development is a matter of screening antigen-specific high-affinity antibody clones from the polyclonal B lymphocyte population. The larger the screening capacity, the better the chance to isolate the best of potentially useful antibodies. SPIN technology employs a two-step screening procedure; a large scale efficient Fluorescence activated cell sorting (FACS) to isolate antigen-specific plasma cells from millions of splenocytes followed by secondary testing of individual plasma cells for their recombinantly expressed antibodies in the supernatant. By isolating antigen-specific plasma cells on FACS, SPIN® technology increases 500-fold the number of antibody clones that can be screened. Therefore, the ability to find the best antibodies, which may be rare and difficult to find with traditional methods, is greatly enhanced.

An example: A Hapten Rabbit Antibody Generated with SPIN® Tech



Cyclophosphamide (Cyc), a small molecule anti-cancer drug used in cancer chemotherapy, has side effects such as vomiting, hair loss and bleeding from the bladder. Monitoring cyclophosphamide metabolism in the blood can guide doctors to adjust to the proper dosage and minimize side effects. An immunological assay was developed to monitor cyclophosphamide levels in the blood. Cyclophosphamide was conjugated to KLH and injected into a rabbit to induce an immune response. After 3 booster shots, rabbit splenocytes were isolated for antibody development with SPIN® technology.

Starting with 2 million splenocytes, we isolated 94 antigen-specific plasma cells. Sixty-five heavy chain and light chain genes from these isolated cells were cloned, of which 61 (93.8%) were shown to be antigen-specific by an ELISA.

Below, we show the EC-50 curves of 4 randomly selected rabbit anti-cyclophosphamide monoclonal antibody producing clones, compared to a positive control (a mouse hybridoma line commercially available).

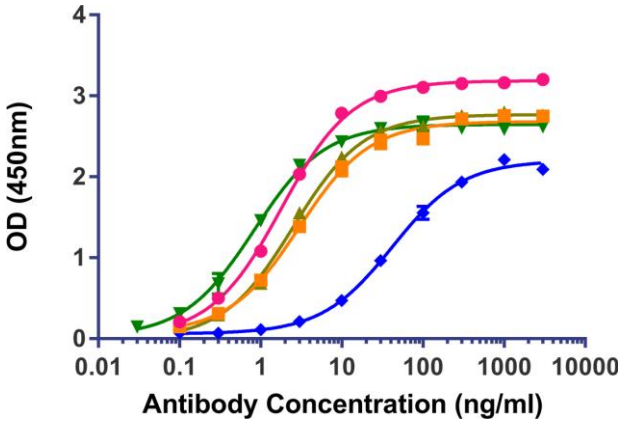


Figure 1. Rabbit anti-Cyc monoclonal antibody generated by SPIN tech. Cyc, a small molecule anti-cancer drug was conjugated to KLH and immunized a rabbit. Immunized rabbit splenocytes were isolated for antibody development with SPIN tech. From sixty-one positive clones, four randomly picked clones were tested with EC50 assay. The positive control antibody (blue) is an anti-cyc antibody from a mouse hybridoma line.

We can safely conclude from this that all four of our randomly selected clones had a greater affinity for Cyclophosphamide than the commercial mouse hybridoma-based standard. Rabbit antibodies have a greater affinity to their antigens in general, and our SPIN technology allows us to screen potential candidates with much greater efficiency.

By combining these advantages — Efficient isolation of antigen-specific plasma cells for mature antibody selection, Single cell amplification for an efficient workflow, and utilizing rabbits for more diverse and higher-affinity antibodies — the SPIN® approach offers scientists a more effective, reliable and reproducible means of quickly obtaining high-quality antibodies. Another advantage of SPIN technology is that it is entirely compatible with producing antibodies from Chickens, or Llamas, each with their own unique and desirable properties.