Rapid Identification of A Diverse Repertoire of High-Affinity SARS-CoV-2 Antibodies

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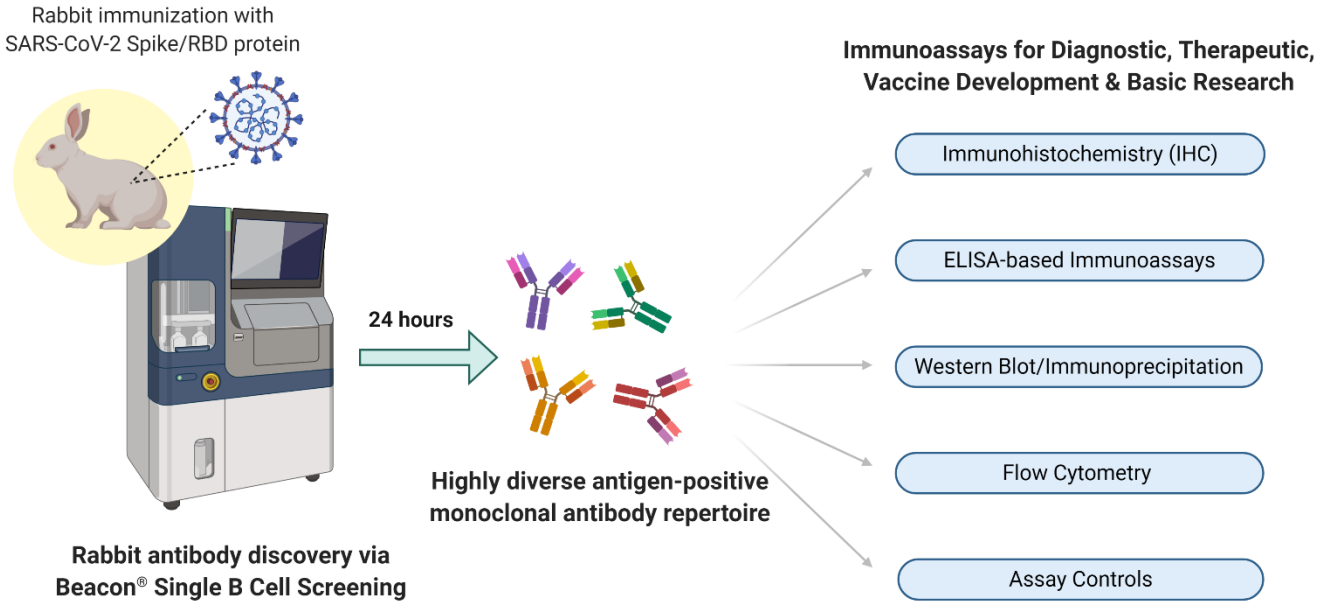
A diverse repertoire of highly specific rabbit monoclonal antibodies (mAbs) with high-affinities were rapidly developed by combining GenScript's **MonoRab™** rabbit mAb service with Berkeley Lights' **Beacon® optofluidic single B cell sorting technology.** The high-throughput automation of the Beacon® platform enabled complete SARS-CoV-2 antibody screening **within just 24 hours**, accelerating a process that would normally take 2 – 3 months using hybridoma technology and synthetic display library methods. Additionally, the Beacon® platform captured a **larger repertoire** of the Spike monoclonal antibodies (mAbs) with a **higher diversity in epitope recognition** compared to mAbs generated using hybridoma technology, thereby providing **multiple epitopes** and thus increasing the chance of **success for optimal assay development**. Consequently, the incredible speed of the Beacon® system and the diverse repertoire of mAbs discovered can greatly benefit researchers seeking to develop sensitive and reliable immunoassays, such as ELISA, immunohistochemistry, flow cytometry, Western blot, immunoprecipitation and much more. Through the rapid generation of a wide selection of diverse, high-affinity mAbs, scientists engaged not only in SARS-CoV-2 research but also in all other research areas can **maximize their success** in advancing towards their research goals.



1. SARS-CoV-2 Rabbit mAb Generation via the Beacon® Platform

The outbreak of the novel coronavirus **SARS-CoV-2** in December 2019 has demanded a great need to quickly generate high-quality antibodies to accelerate therapeutic and diagnostic development. The **Spike (S)** protein of SARS-CoV-2 engages the ACE2 receptor of the host cell via its **receptor binding domain (RBD)** and undergoes conformational changes that enable the fusion of the viral membrane with the host cell membrane, and ultimately viral entry into the host cell[3]. As a result, the S protein is a key target for the development of therapeutics, vaccines, and diagnostic reagents[1,4].

The **Beacon® single B cell screening platform**, coupled with **GenScript's MonoRab™ rabbit mAb generation platform**, greatly accelerates the discovery of a diverse mAb repertoire, by harnessing the cutting-edge technology of Beacon® and capitalizing on the unique rabbit immune system and its ability to generate antibodies of high specificity and affinity. Within 24 hours, we completed immune cell isolation and enrichment, single cell sorting, as well as on-chip binding and blocking screening (**Figure 1, Appendix**), a process that would have otherwise taken months using the traditional ELISA-based hybridoma and synthetic DNA library antibody screening methods (**Table 1**).

GenScript possesses a wide range of technologies that help maximize the success of generating high-quality antibodies for your research application. OptimumAntigen™, our antigen design tool harnesses advanced computational algorithms to comprehensively evaluate and optimize antigen characteristics for maximal antigenicity. Additionally, our immunization technologies like ImmunoPlus™ and proprietary adjuvants help stimulate a robust immune response within immunized hosts, enabling high-affinity antibody generation.

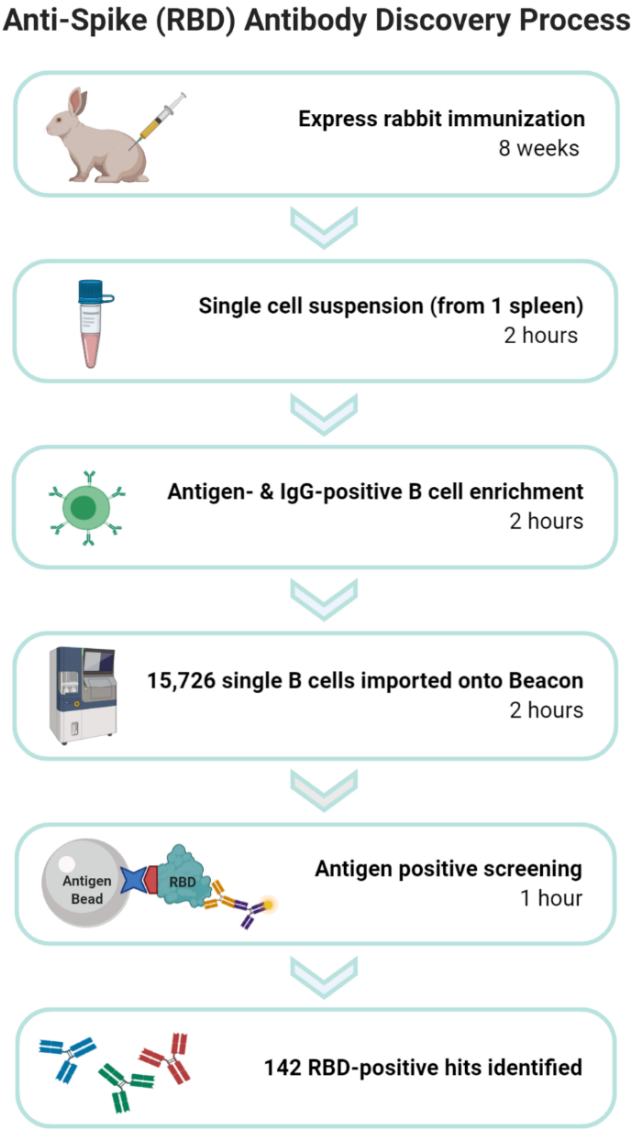


Figure 1: Anti-Spike (RBD) mAb discovery workflow and the corresponding timeline. Within 24 hours, cells isolated from the spleen of an immunized rabbit were enriched for ASCs, and over 15,000 cells were imported onto a 14k Optoselect Chip on the Beacon® platform and subjected to in-chip screening to identify mAb clones positive for RBD binding. In total, 142 antigen-positive hits were identified for downstream evaluation.

Table 1: Advantages of the Beacon platform for antibody discovery.

	Hybridoma	Library	Beacon® Platform
Antibody Source	Immunized host	Synthetic (Naïve/immunized)	Immunized host
Technology	Traditional	Newer	Latest
Time Screening	3 months	1 - 2 months	1 day
Natural cognate chain pairing	Y	N	Y
Affinity	High	Low to High	High
Antibody Diversity	Low-Medium	Low-Medium (Biased)	Highest

2. Rapid Screening and Characterization of Spike/RBD Antibodies within 24 hours

Altogether, 15,726 single B cells were subject to automated screening using an antigen-specific bead assay to identify mAbs positive for RBD-binding that were being secreted from their corresponding B cells. In total, antibody screening and selection resulted in 142 antigen-positive mAb hits (**Appendix**). **The entire process, including the import, screening, and identification of RBD-positive hits for export, took less than 24 hours.** In contrast, antibody screening methods typical of hybridoma and synthetic display library methods usually take several months (**Table 1**).

2.1. Rabbit mAbs display high-affinity towards the Spike/RBD protein

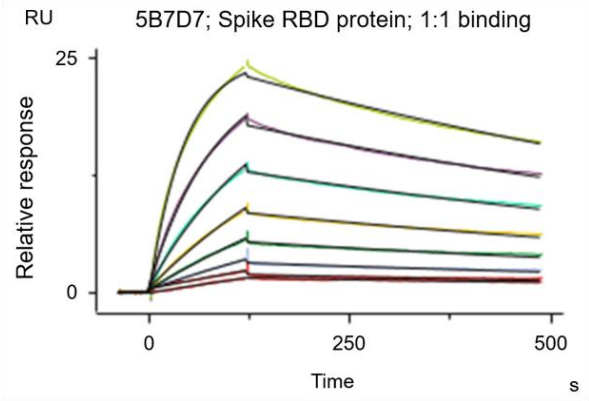
Of the 142 antigen-positive hits identified, 60 were exported for sequencing. As expected, evaluation of the top rabbit mAbs using Biacore revealed that rabbit mAbs possessed higher affinities than rodent mAbs towards the Spike/RBD protein. Most rabbit mAbs displayed 10–100 fold higher affinity (K_D ~10^{-10}–10^{-11} range) in comparison to the top rodent mAbs (K_D ~10^{-9} range) (**Figure 2**).

GenScript also offers a range of specialized services like antibody labeling, custom ELISA kit development, scale up production, and more!

A)

Species	Platform	Clone ID	K_D (M)
Rabbit	Beacon	BS-R2B2	4.94E-09
Rabbit	Beacon	BS-R2B12	2.70E-10
Rabbit	Beacon	BS-R2B17	1.65E-10
Rabbit	Beacon	BS-R2B30	7.57E-10
Rabbit	Beacon	BS-R1B8	2.17E-09
Rabbit	Hybridoma	39 G6	3.05E-11
Mouse	Hybridoma	6D11F2	3.52E-09
Mouse	Hybridoma	11D5D3	1.89E-09
Mouse	Hybridoma	5E10G8	1.05E-09
Mouse	Hybridoma	5B7D7	4.60E-09
Mouse	Hybridoma	10G6H5	5.73E-09

B)



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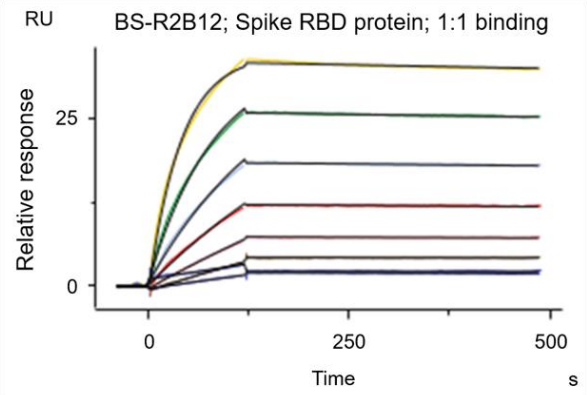

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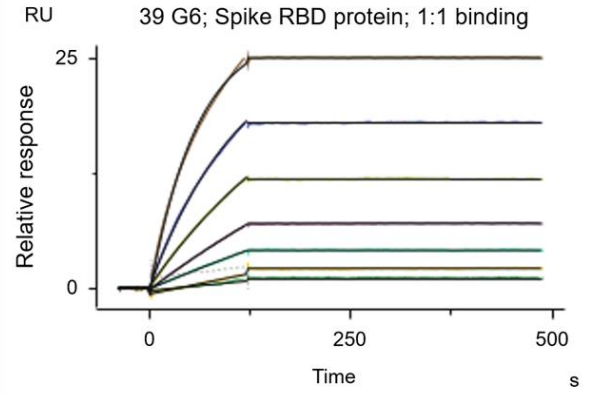

Figure 2: Rabbit mAbs show higher affinity towards the Spike/RBD compared to mouse mAbs. (A) List of KD values of selected top mouse and rabbit Spike/RBD antibodies as measured by Biacore 8K (GE healthcare) (B - D) Biacore antigen-binding response curves for the top mouse clone 5B7D7 (B) the rabbit clone BS-R2B12 discovered using the Beacon platform (C), and the rabbit clone 39 G6 using the MonoRab™ hybridoma approach (D). (Note: All rabbit mAbs discovered using the Beacon® platform are labeled with the prefix 'BS', e.g. BS-R2B2, BS-R2B12, BS-R2B17, etc.)

2.2. Epitope binning reveals high Spike/RBD rabbit mAb diversity

In addition, we subjected several rabbit mAb clones to epitope binning and found that the rabbit mAbs from the Beacon® platform were distributed across 4 different epitope bins. In contrast, using the MonoRab™ rabbit hybridoma approach, only 1 epitope bin was occupied with several rabbit mAb clones (**Figure 3A**). While more hybridoma-derived mAbs certainly need to be evaluated for a fair comparison, this observation is in agreement with what the broader scientific community has observed – a more diverse antibody repertoire can be discovered using the Beacon® platform at an efficiency that cannot be achieved via hybridoma technology[5].

A)

Epitope Bin	Spike/RBD Rabbit mAb Clone ID			
1	BS-R2B12	BS-R2B16	BS-R2B30	
2	BS-R2B17	BS-R2B27	BS-R2B50	
3	BS-R2B2	4G6	12D3	39 G6
4	BS-R1B8			

B)

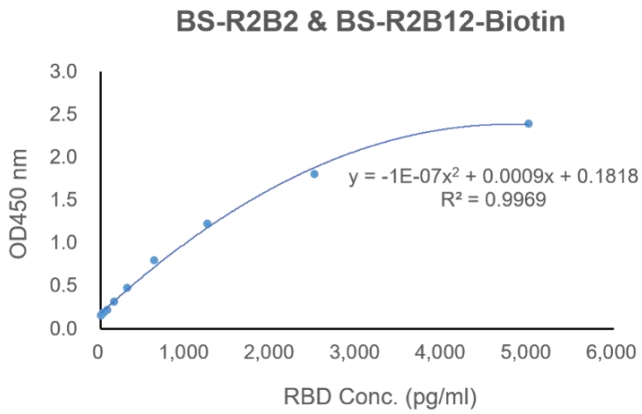

Figure 3: Development of a highly sensitive sandwich ELISA kit for Spike/RBD detection, based on a greater rabbit mAb diversity. (A) Epitope binning data reveals that top Beacon-derived rabbit mAbs fall into 4 different epitope bins, while top rabbit mAb hybridoma clones (red font) all fall within the same epitope bin. (B) Sandwich ELISA assay development for Spike/RBD using Beacon rabbit mAb clones BS-R2B2 & BS-R2B12-Biotin, the assay sensitivity was determined to be 20 pg/ml.

The generation of diverse mAbs that recognize different epitopes on a target protein are critical for a variety of reasons (**Table 2**). The development of sensitive and specific immunoassays, such as immunohistochemistry (IHC), ELISA, flow cytometry, or Western blot, hinges on the availability of a variety of high-quality mAbs that researchers can evaluate in-house to identify and qualify the top candidates that best work in their assays. In addition, specialized immunoassays such as sandwich ELISA, rely on antibody diversity due to the need for mAb pairs that recognize and bind to unique epitopes on an antigen without sterically interfering with each other. By obtaining as diverse an antibody repertoire as possible, opportunities for researchers to identify the best and most compatible mAb pairs are maximized. In fact, from the Spike/RBD mAbs we identified, we developed a highly sensitive sandwich ELISA assay using clones BS-R2B2 and biotinylated BS-R2B12, with a sensitivity for the Spike/RBD protein of 20 pg/ml (**Figure 3B**). Furthermore, in industrial therapeutic and diagnostic research where antibody generation becomes an integral part of the discovery process, generating as diverse an antibody population as possible is essential. Not only does maximizing the diversity of mAbs discovered increase the potential of identifying the ideal therapeutic or diagnostic mAb, but it also maximizes the chance of identifying a unique target binding epitope. This may be especially important for mAbs intended for commercial use, where novel antibody sequences for therapeutics and/or diagnostics are valuable intellectual property. The Beacon® platform's ability to identify a bigger antibody repertoire provides researchers with benefits that cannot be matched using hybridoma technology.

Table 2: Key benefits of identifying an antibody repertoire with high epitope diversity.

Antibody application	Benefits of Epitope Diversity
General Immunoassays *e.g. IHC, ELISA, Western blot, Immunoprecipitation, flow cytometry*	- Permits cross-validation of mAb specificity towards target - Increases options for identifying top mAb(s) specific to research application - Enables antibody pairing (sandwich ELISA), and maximizes options for identifying best antibody pair - Increases opportunity for identifying mAb(s) towards challenging epitopes
mAb discovery *e.g. Therapeutic/diagnostic mAb discovery with intent to commercialize*	- Increases chances of identifying mAb towards unique target epitope (facilitates patent filing and IP protection) - Identification of suitable mAb controls independent of epitope of interest

3. Summary

The Berkeley Lights Beacon® Single B Cell Sorting platform is a highly efficient method to accelerate the discovery of a diverse array of high-quality rabbit monoclonal antibodies critical for immunoassay development across all research fields and applications, from therapeutic, diagnostic and vaccine development as well as basic research. Top mAbs discovered from this project possessed high affinities towards the Spike RBD of SARS-CoV-2 and recognized 4 unique epitopes, which enabled mAb pairing and the subsequent development of a sensitive sandwich ELISA assay.

By coupling the unique advantages of the rabbit immune system with the cutting-edge Beacon® single B cell sorting platform, scientists can maximize their opportunity to rapidly identify high-quality, diverse mAbs critical for advancing their research at a speed and diversity that simply cannot be achieved using conventional hybridoma and display library mAb generation technologies. At the same time, the advanced technology and automation of the Beacon® system reduces the amount of labor required and minimizes human error.

GenScript is currently the only vendor offering rabbit mAb discovery via the Beacon® platform, a testament to our expertise in rabbit mAb discovery and generation. Start your antibody discovery project with us today to achieve the most diversified antibody repertoire and successfully capture all opportunities to advance your research!

References

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3. Shang, J. *et al.* Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. U. S. A.* (2020). doi:10.1073/pnas.2003138117
4. Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* (2020). doi:10.1016/j.cell.2020.02.058
5. Winters, A. *et al.* Rapid single B cell antibody discovery using nanopens and structured light. *MAbs* **11**, 1025–1035 (2019).

All antibodies mentioned in this application note are ideal reagents for a wide variety of research applications. To obtain further information on these antibodies or any of our services, please e-mail antibody@genscript.com, or visit us here.

Appendix

Workflow for Single B Cell Antibody Discovery via the Beacon® Platform

Phase I: Animal Immunization
The host species (rabbit or rodent) for mAb generation is immunized with a target immunogen to stimulate antibody production. Depending on the immunization strategy, after 4 - 10 weeks, the animal is sacrificed and tissues containing antibody secreting cells (ASC) such as the spleen, lymph nodes and blood are harvested.

Phase II: Antibody Secreting Cell (ASC) Enrichment
Antigen-specific B cells, which eventually mature into antibody-producing plasma B cells, are isolated by way of antigen or CD138 cell surface marker enrichment and stimulation.

Phase III: Single B Cell Screening via the Beacon® Platform
Enriched ASCs are then loaded onto the Beacon® platform, and in a fully automated process, light is used to import individual B cells into a NanoPen™ chamber located on a 14k Optoselect™ chip. Once cells are loaded onto the chip, a variety of in-chip screening and selection assays can be conducted to identify the specific B cells that are secreting the desired antibody.

Phase IV: Single B Cell Sequencing
Once positive hits have been determined, the selected cells are then exported from the Beacon® system for sequencing. RNA is isolated from each B cell, and RT-PCR is conducted to acquire cDNA. The VH and VL regions of each antibody are then amplified for sequencing and analysis.

Phase V: Small-Scale Recombinant Antibody Expression
Selected antibodies are then cloned into expression vectors and expressed recombinantly at small-scale for further screening, affinity and functional testing before scale-up expression.

The general experiment workflow using the Beacon® Single B Cell Sorting platform for rabbit monoclonal antibody discovery.

Antibody Discovery via Beacon® Single B Cell Screening

Single B cell sorting technology is a popular microsystem-based screening method that is increasingly being adopted throughout the field of antibody discovery. The Beacon® platform by Berkeley Lights harnesses the latest in optofluidics technology, and combines the isolation, high-throughput screening and evaluation of B cells on a chip to accelerate the discovery of a highly diverse antibody repertoire.

- Cutting-edge technology **maximizes chances** of identifying your ideal mAb
- **Fast track** antibody discovery and shorten your time investment
- Streamlined, **automated workflow** and platform
- Discover **high-diversity**, **high-specificity** reagent antibodies

Service Specifications at a Glance

Milestones	Description	Timeline
Phase I Preparation	Animal immunization and starting material preparation	5-9 weeks
Phase II Screening and on-chip assay via Beacon	Single B cell sorting and on-chip assay to find positive clones	1 week
Phase III Antibody Production	Variable domain sequencing and recombinant antibody expression	4 weeks

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