InvivoGen insight

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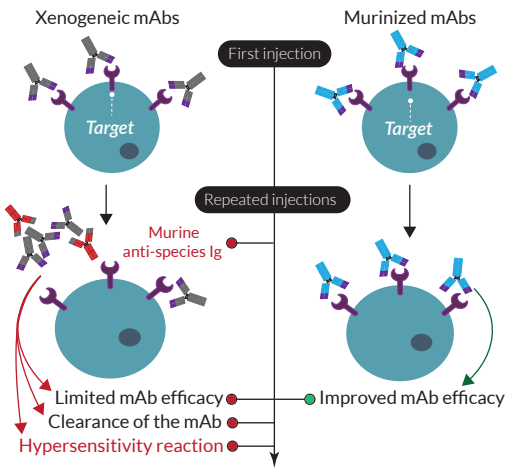
Inside this issue

In this *insight* newsletter, we highlight two unrelated product ranges. The first is a growing collection of monoclonal antibodies optimized for *in vivo* mouse studies. The second is a new series of COVID-19 related cell lines, specifically designed for developing novel therapeutics against SARS-CoV-2.

In 1985, the FDA approved the first monoclonal antibody (mAb) for clinical use. This mAb was an anti-CD3 antibody for the treatment of organ allograft rejection[1]. Being of mouse origin, all patients administered with this mAb developed a human anti-mouse immunoglobulin (Ig) antibody (HAMA) response, significantly limiting its use[2]. This response leads to the inactivation and clearance of the circulating mAb, causing a loss of treatment efficacy, and in some cases, severe hypersensitivity reactions[3]. To overcome this limitation, the CD3 mAb was humanized by engineering the complementarity determining regions (CDRs) into a human backbone[4]. Currently, all clinically-approved mAbs are either humanized or 'fully' human through the use of various technologies (e.g. humanized mice)[1].

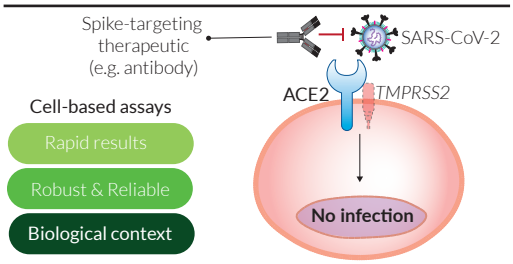
A similar unwanted 'anti-antibody' response has been reported in mice upon repeated administration of non-murine, or xenogeneic (e.g. rat/hamster) mAbs. This mouse immune response not only clears the administered mAb, but can also cause a lethal hypersensitivity reaction[5-7]. The use of xenogeneic mAbs for *in vivo* preclinical research can have significant effects on the assessment of optimal dosing, timing, and repeated injections for the initial in-human trials. Therefore, similar technology used to optimize human mAbs has been applied to mAbs for *in vivo* mouse research. Firstly, mAbs can be 'murinized' through the complete replacement of the non-murine constant regions with mouse Ig sequences, resulting in chimeric mAbs. Importantly, this type of engineering has been shown to not only prolong the circulation of the administrated mAb but also significantly decrease the hypersensitivity reaction upon repeated injections[7]. Further murinization can be achieved through the murine grafting of the variable CDRs. However, similar to the process for humanization, it is quite complex, difficult to achieve, and can generate a mAb with lower affinity. Ideally, fully murine mAbs can be generated by targeting a gene of interest in knockout (KO) mice. However, this is difficult if the target knock out is embryonically lethal.

Through the use of recombinant technology, mAbs for *in vivo* research can be significantly improved through the murinization of the backbone and/or the replacement of the isotype for optimal effector function.



The global spread of COVID-19 has led to an unprecedented worldwide effort to rapidly develop specific therapeutics, including neutralizing antibodies, inhibitory small molecules, and vaccines. The majority of research has been dedicated to finding ways to inhibit viral entry into host cells by blocking the interaction between the SARS-CoV-2 antigenic Spike protein and the host receptor ACE2.

A crucial step in developing successful COVID-19 treatments is the validation of their efficacy using rapid, reliable, and robust platforms. To this end, various types of *in vitro* assays have been developed. Among them, cell-based assays (CBAs) present the key advantage of providing a more relevant *in vivo* biological context than biochemical assays and thus, reduce the number of animal tests needed, while accelerating the drug discovery process.



InvivoGen has developed a unique collection of cell lines, that are high-throughput-ready, and specifically engineered to be permissive to SARS-CoV-2 infection. These cell lines derive from two different cellular backgrounds, the commonly used HEK293 cell line, and the more COVID-19 relevant A549 lung cell line. The HEK293 cells have been modified to express ACE2, while the A549 cells have been transfected with both ACE2 and the Spike-cleaving protease TMPRSS2 for optimal permissivity. The expression of ACE2 in these cells has been confirmed in flow cytometry binding assays using a recombinant Spike-(S1)-Fc protein. To validate the permissivity of these cell lines, inhibition assays were performed using Spike-pseudotyped GFP lentiviral particles and various potential inhibitors, including neutralizing mAbs. Importantly, the data obtained with particles expressing two variants of the spike protein (D614 or G614), have confirmed the reliability and reproducibility of these CBAs.

Our COVID-19 related cell lines, as well as the various tools used in their development, are now available.

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Mouse Anti-Mouse InvivoFit™ mAbs

Monoclonal antibodies (mAbs) for *in vivo* use are typically produced in other species (e.g. rat and hamster) and hence, they are recognized as foreign when injected into mice. Upon repeated injections of these non-murine (i.e. xenogeneic) antibodies, the mice mount an immune response, producing anti-species antibodies. This leads to unwanted effects such as limited efficacy of the administered mAb, and eventually a deadly pro-inflammatory hypersensitivity response. InvivoGen has specifically engineered a collection of recombinant mouse anti-mouse mAbs to limit their *in vivo* immunogenicity.

Murinization for reduced toxicity *in vivo*

Murinization of a mAb requires the replacement of sequences of non-murine origin with murine counterparts. InvivoGen has murinized a series of mAbs by replacing the non-murine constant regions of anti-human/rat/hamster mAbs with murine IgG sequences. Therefore, our mouse anti-mouse mAbs display:

- Reduced anti-species response
- Prolonged circulation of administered mAb
- Reduced toxicity and/or hypersensitivity reaction

Upon repeated injection of xenogeneic mAbs, mice mount a dose-dependent immune response initially directed against the constant region. Ultimately, this response is directed against the whole molecule. The production of mouse anti-species antibodies removes the administered mAb from circulation, thereby reducing its *in vivo* efficacy. Furthermore, this immunogenicity can lead to fatal hypersensitivity reactions. In particular, this response has been noted in studies of xenogeneic mAbs targeting immune checkpoints (e.g. PD-1/PD-L1[5] and GITR[6,7]), with no unwanted response seen for murine mAbs (i.e. Anti-mCLTA4). In these studies, upon the 3rd or 4th injection of the mAb, the mice exhibited signs of hypersensitivity-associated distress, such as lethargy, piloerection, dyspnea, and a rapid drop in body temperature[5-7] (*see below for in-house data*).
Importantly, mAb murinization can prolong circulation of the administered mAb and it has been shown to reduce hypersensitivity reactions[7].

LIMITED RISK OF A HYPERSENSITIVITY REACTION WITH A MURINIZED ANTIBODY

To demonstrate the induction of a non-species response in mice, we compared our effectorless human Atezolizumab-derived Anti-PD-L1 mAb against its murinized version (*see Fig 1*). Approximately 30 minutes after the 4th injection, a drop in body temperature (red line), along with other symptoms of a hypersensitivity reaction, were observed in the non-murine mAb-treated group. With intervention, (e.g. external heating) these mice survived. On the other hand, the murinized mAb-treated group maintained their body temperature (purple line) comparable to the negative control (blue line), with no signs of a hypersensitivity reaction.

Recombinant production for increased quality

Most mAbs on the market for *in vivo* research are produced in hybridomas, which have a number of drawbacks including, the exchange of light chains and cellular derivations[8]. InvivoGen uses recombinant technology to overcome these issues (*see Fig 2*). Additionally, this technology allows us to replace the original isotype with an optimal format for proven increased efficacy *in vivo*[9].

- Lot-to-lot structure consistency
- Optimized isotype format

Our mouse anti-mouse mAbs are expressed and produced in CHO cells (virus-free status confirmed), ensuring reliability and reproducibility.

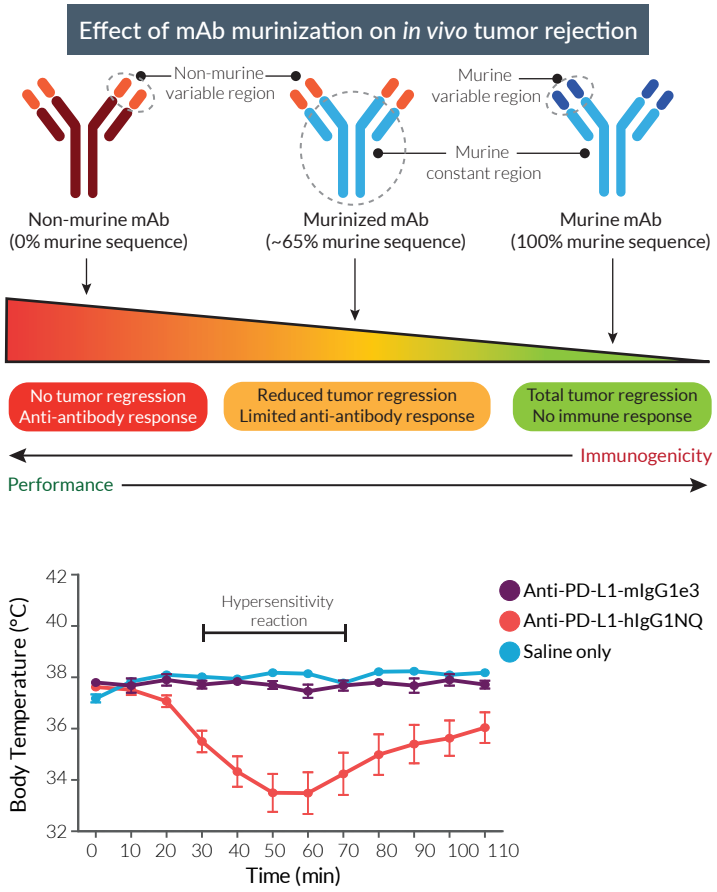
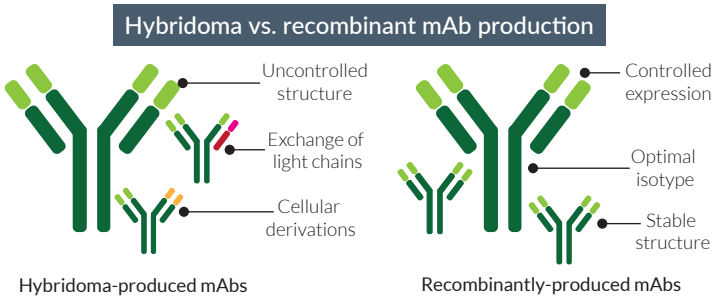


Fig 1: Body temperature comparison between human and murinized Anti-PD-L1 mAb after IP4. BALB/c mice aged 10 weeks were challenged subcutaneously with 2 x 10^5 CT26 cells. After 8 days, Anti-PD-L1-mIgG1e3 (murine) or Anti-PD-L1-hIgG1NQ (human) was administered intraperitoneally (IP) into the mice in sterile PBS (200 µg/mouse). IP injections were then performed twice a week for 3 weeks. After IP4, rectal temperature measurements were taken every 10 minutes for all groups. Data are presented as mean ± SEM.



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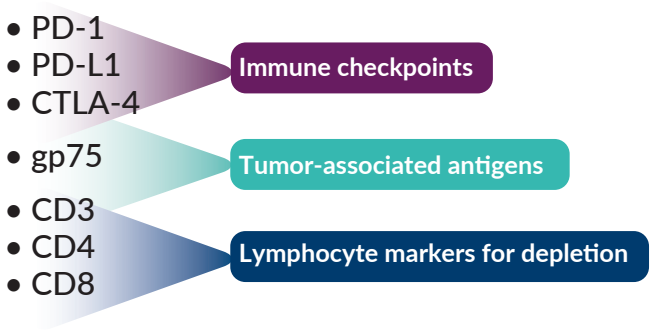
InvivoGen offers an expanding collection of high-quality mouse anti-mouse mAbs that target various immune checkpoints (ICs), tumor-associated antigens, or lymphocyte markers for cell depletion listed below.



All the mAbs in our mouse anti-mouse collection have been produced by recombinant technology. For some of the mAbs that are of murine origin (e.g. Anti-mCTLA4), we have replaced the original isotype with a format (i.e. IgG2b vs IgG2a) for optimal *in vivo* efficacy[9] (*see Fig 3*). For other anti-human/rat/hamster mAbs, we have replaced the entire non-murine constant region with mouse IgG sequences. Furthermore, we have ensured that the same effector function is maintained. For example, the murine IgG1e3 isotype of our Anti-PD-L1 mAb, which derives from the effectorless PD-L1 mAb (Atezolizumab), has been engineered to include a mutation that results in a complete loss of effector function.

To ensure consistently high-quality mouse anti-mouse mAbs for your research, InvivoGen performs rigorous quality control.

- Highly pure (>95%)
- Low aggregation (<5%)
- Sterile
- Lot-to-lot reproducible target binding

Our collection is provided in an **InvivoFit™ grade**, a high-quality standard specifically adapted for *in vivo* studies. This standard means that our mouse anti-mouse mAbs are guaranteed sterile and endotoxin-free (<1 EU/mg).

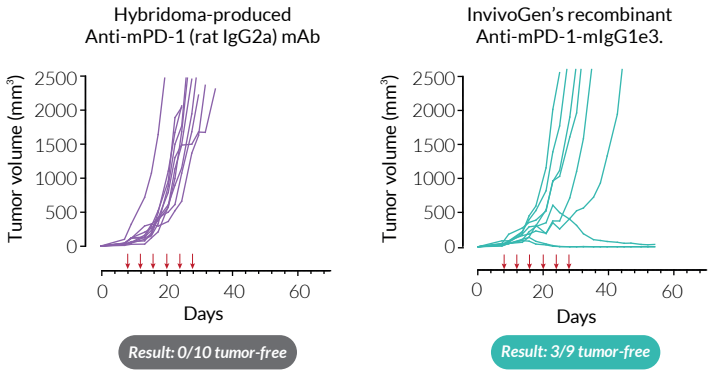


Fig 2: Tumor growth after treatment with a hybridoma-produced or recombinantly-produced Anti-PD-1 mAb. BALB/c mice aged 10 weeks were challenged subcutaneously with 2 x 10^5 CT26 cells. After 8 days, InvivoGen's Anti-mPD-1-mIgG1e3 or a hybridoma-produced Anti-PD-1 mAb was administered intraperitoneally (IP) into the mice in sterile PBS (200 µg/mouse). Following this, IP injections were performed twice a week for 3 weeks (red arrows). Tumor growth was monitored for 60 days.

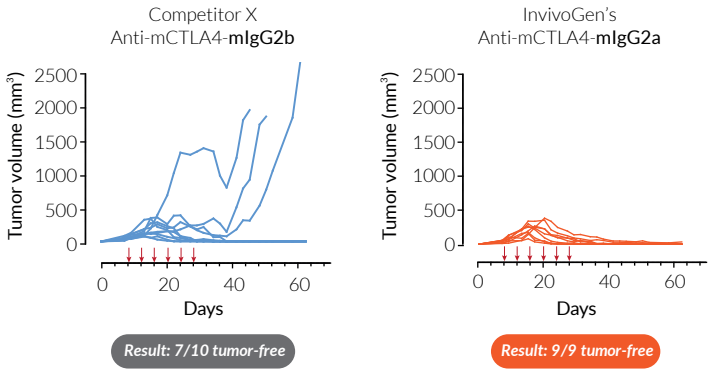


Fig 3: Tumor growth after treatment with a murine Anti-CTLA4-mIgG2b and Anti-CTLA4-mIgG2a mAb. BALB/c mice aged 10 weeks were challenged subcutaneously with 2 x 10^5 CT26 cells. After 8 days, InvivoGen's Anti-mCTLA4-mIgG2a or Competitor X's Anti-mCTLA4-mIgG2b mAb was administered intraperitoneally (IP) into the mice in sterile PBS (200 µg/mouse). Following this, IP injections were performed twice a week for 3 weeks (red arrows). Tumor growth was monitored for 60 days.

PRODUCT	DESCRIPTION	QTY	CAT. CODE
***In vivo* depletion mAbs**			
Anti-mCD3-mIgG2a InvivoFit™ NEW	145-2C11-derived mouse mAb against murine CD3	1mg / 10 mg	mcd3-mab10-1/-10
Anti-mCD4-mIgG2a InvivoFit™ NEW	GK1.5-derived mouse mAb against murine CD4	1mg / 10 mg	mcd4-mab10-1/-10
Anti-mCD8-mIgG2a InvivoFit™ NEW	YTS169.4-derived mouse mAb against murine CD8	1mg / 10 mg	mcd8-mab10-1/-10
***In vivo* immune checkpoint blocking mAbs**			
Anti-mCTLA4-mIgG2a InvivoFit™	9D9-derived mouse mAb against murine CTLA-4	1mg / 10 mg	mctla4-mab10-1/-10
Anti-mPD-1-mIgG1e3 InvivoFit™	RMP1-14-derived mouse mAb against murine PD-1	1mg / 10 mg	mpd1-mab15-1/-10
Anti-PD-L1-mIgG1e3 InvivoFit™	Murinized atezolizumab mouse mAb against PD-L1	1mg / 10 mg	pdl1-mab15-1/-10
***In vivo* tumor-associated antigen mAbs**			
Anti-mgp75-mIgG2a InvivoFit™	TA99-derived mouse mAb against murine gp75	1mg / 10 mg	mgp75-mab10-1/-10
***In vivo* isotype controls**			
Anti-β-Gal-mIgG1e3 InvivoFit™	Mouse IgG1e3 isotype control	1mg / 10 mg	bgal-mab15-1/-10
Anti-β-Gal-mIgG2a InvivoFit™	Mouse IgG2a isotype control	1mg / 10 mg	bgal-mab10-1/-10

InvivoGen offers **bulk quantities** of mAbs in our mouse anti-mouse collection **upon request**.
Please contact us for more information.

To learn more about our mouse anti-mouse mAb collection visit:

www.invivogen.com/mouse-anti-mouse-mabs

COVID-19-Related Cell Lines

SARS-CoV-2 (2019-nCoV), is a novel β-coronavirus, and the causative agent of the global COVID-19 pandemic. InvivoGen offers an expanding collection of cell lines to study this virus. These cells derive from two cellular models, the commonly used human embryonic kidney 293 (HEK293) cell line and the biologically relevant human A549 lung carcinoma cell line.

- HEK-Blue™ hACE2 Cells NEW
- A549-hACE2-TMPRSS2 Cells NEW

- Verified over-expression of SARS-CoV-2 host receptors
- Functionally validated in infection assays
- Designed for screening SARS-CoV-2 inhibitors

HEK-Blue™ hACE2 and **A549-hACE2-TMPRSS2 cells** express high levels of human (h)ACE2 at their cell surface, making them permissive to SARS-CoV-2 infection. The addition of TMPRSS2 to ACE2-expressing HEK293 cells does not increase their permissivity, whereas, its addition to ACE2-expressing A549 cells (**A549-hACE2 cells**) significantly improves their infectivity (*see Fig 1*).
These cell lines are high-throughput-ready and ideal for screening novel small molecule inhibitors or neutralizing antibodies isolated from convalescent COVID-19 patients that block the interaction between SARS-CoV-2 and the host cell (*see Fig 2*).

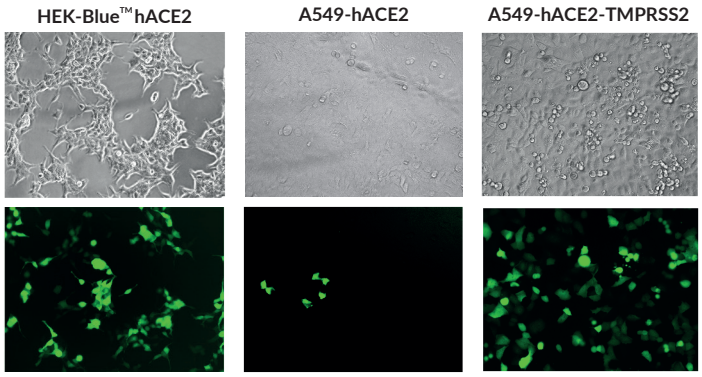


Fig 1: Infection by SARS-CoV-2 Spike pseudotyped lentiviral particles. HEK-Blue™ hACE2, A549-hACE2, and A549-hACE2-TMPRSS2 cells were cultured in the presence of SARS-CoV-2 **Spike-D614** (HEK293) or **Spike-G614** (A549) **pseudotyped** GFP lentiviral particles. After 72 hours, the transduction efficiency of the Spike-pseudotyped GFP particles was evaluated by fluorescence microscopy.

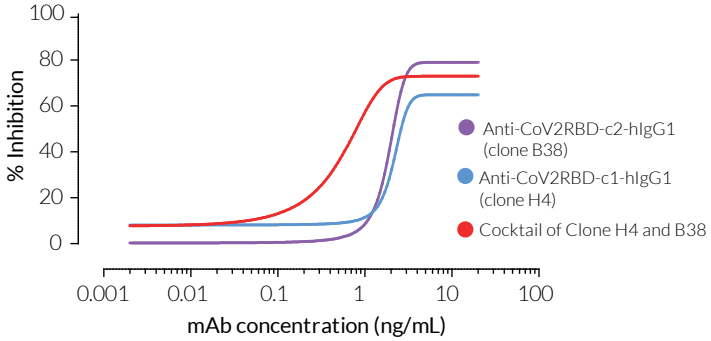
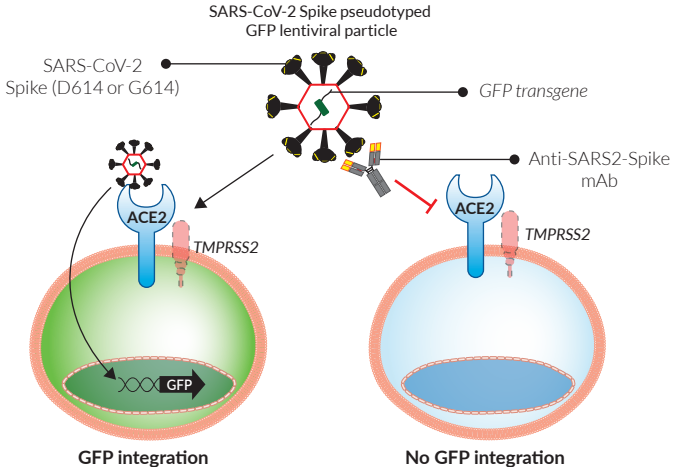


Fig 2: Neutralization ability of Anti-SARS-CoV-2 Spike-RBD mAbs. SARS-CoV-2 spike-RBD targeting mAbs (**clones H4 & B38**) were incubated with Spike (G614)-pseudotyped lentiviral particles for 1 hour at 37°C. Following this, **HEK-Blue™ hACE2 cells** were added and incubated for 72 hours. Infection (GFP fluorescence) was then measured using flow cytometry. Data are presented as % inhibition of infection compared to a no antibody control.

Application of cell-based assays for SARS-CoV-2 research



PRODUCT	QTY	CAT. CODE
HEK-Blue™ hACE2 Cells	3-7 x 10^6 cells	hkb-hace2
A549-hACE2-TMPRSS2 Cells	3-7 x 10^6 cells	a549-hace2tpsa
A549-hACE2 Cells (control)	3-7 x 10^6 cells	a549-hace2

COMING SOON

A549 cells express a functional RNA sensing pathway, making them a useful cellular model for studying the host response to SARS-CoV-2 infection. We have developed a series of **A549-Dual™ hACE2-TMPRSS2 cells** that express NF-κB and IRF-dependent SEAP and luciferase reporter genes, respectively. In these cells, the innate receptors of RNA viruses, RIG-I or MDA5, have been knocked out. Therefore, they are ideal for understanding the role of these receptors in SARS-CoV-2 infection.

- **A549-Dual™ hACE2-TMPRSS2**
- **A549-Dual™ KO-RIG-I hACE2-TMPRSS2**
- **A549-Dual™ KO-MDA5 hACE2-TMPRSS2**

OTHER PRODUCTS YOU MAY NEED

pLV-SARS2-S-d19	Spike D614 pseudotyping vector
pLV-SARS2-S-d19 (D614G)	Spike G614 pseudotyping vector
Anti-CoV2RBD-c1-hIgG1	SARS-CoV-2 mAb (clone H4)
Anti-CoV2RBD-c2-hIgG1	SARS-CoV-2 mAb (clone B38)

For a complete list of SARS-CoV-2 related research tools visit:

www.invivogen.com/covid-19

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