InvivoGen insight

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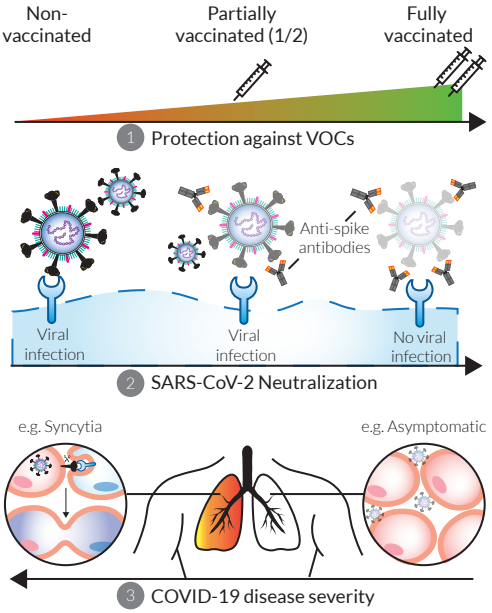
The race against SARS-CoV-2 variants

The ongoing COVID-19 pandemic has had a devastating global impact on public health, the economy and society. Effective vaccines have been developed at an unprecedented rate in an effort to curb the spread of SARS-CoV-2. Currently, there are 4 main types of COVID-19 vaccines that have been approved for use or are under development, mRNA-, viral vector-, protein subunit-, and inactivated viral-based vaccines[1]. The majority of the global vaccination effort is centered on the mRNA-based vaccines BNT162b2 and mRNA-1273 developed by Pfizer-BioNTech and Moderna, respectively, and viral vector-based vaccines, ChAdOx1 nCoV-19 and Ad26.COV2.S, developed by AstraZeneca and Johnson & Johnson/Janssen, respectively. They all encode the SARS-CoV-2 Spike (S) protein and aim to prevent disease primarily by triggering the production of neutralizing antibodies that block the S protein and thus, inhibit viral entry[2]. These vaccines were designed based on the S protein from the original SARS-CoV-2 strain circulating early in the pandemic. However, several variants of concern (VOC) and variants of interest (VOI) have emerged[2-4]. Therefore, the question is: will these variants jeopardize the efficacy of the newly introduced vaccines?

Currently, the circulating VOC were first identified in the United Kingdom (B.1.1.7, Alpha), South Africa (B.1.351, Beta), Brazil (P.1, Gamma), and India (B.1.617.2, Delta). Further VOI were first identified in the United States (B.1.429, Epsilon and B.1.526, Iota) and India (B.1.617.1, Kappa) (*see table*)[4]. VOC and VOI are characterized by mutations and/or deletions within the S coding region. Several of these have been shown to either increase transmissibility by augmenting binding to the host receptor ACE2 (e.g., D614G and N501Y), and/or aid in immune evasion by altering epitopes of neutralizing antibodies (e.g., K417N/T, L452R, and E484K/Q)[3]. Many of these mutations are observed within the receptor binding domain (RBD) of the S protein. This is quite concerning considering that serological analyses of individuals infected with SARS-CoV-2 have indicated that the major activity of serum antibodies targets the RBD[5].



SARS-CoV-2 variant (V) nomenclature

InvivoGen	PANGO	WHO[4]
Original	(D614)	-
V1	(G614)	-
V2	B.1.1.7	Alpha
V3	B.1.351	Beta
V4	B.1.429	Epsilon
V5	P.1	Gamma
V6	B.1.526	Iota
V7	B.1.617.1	Kappa
V8	B.1.617.2	Delta

To date, research has indicated that the approved vaccines are effective against the emerging variants. However, their efficacy varies depending on several factors including, the variant, the vaccine, and if the individual is partially (1 dose for the 2-dose regimes) or fully vaccinated (2 doses for the 2-dose regimes)[6,7]. Generally, full vaccination with either BNT162b2, mRNA-1273 or ChAdOx1 provides a significant level of protection against symptomatic infections/hospitalizations caused by all VOC identified so far[8]. Therefore, one of the main global health priorities is to continue monitoring the effectiveness of the existing vaccines and decide whether additional doses or even adaptations with the mutated Spike are needed to combat the emerging variants.

Vaccines are not fully protective and there will always be breakthrough cases[9,10]. Thus far, the vaccines seem to be an effective countermeasure against severe COVID-19 disease, where the viral load is high and complications can occur. In such cases, a phenomenon known as 'Spike-driven syncytia' has been observed[11]. Besides its ability to drive fusion of viral and cellular membranes, the S protein is able to drive the fusion of neighboring cells, resulting in the formation of multinucleated giant cells, so called syncytia. This phenomenon has been associated with chronic tissue damage in previous coronavirus outbreaks (i.e. SARS and MERS) and has been frequently observed in the autopsies of individuals with severe COVID-19[11,12]. Syncytia formation is believed to contribute to COVID-19 severity. However, comprehensive research is still needed to fully understand VOC pathogenesis.

Our understanding of SARS-CoV-2 has grown at an extraordinary rate throughout the pandemic. Nonetheless, vaccines can only go so far, and urgent research must continuee worldwide to develop targeted therapeutics against SARS-CoV-2.

1. Noh, J.Y. *et al.* 2021. *Signal Transduct Target Ther* 6, 203. doi:10.1038/s41392-021-00623-2. **2. Altmann, D.M. *et al.* 2021.** *Science* 371, 1103-1104. doi:10.1126/science.abg7404. **3. Harvey, W.T. *et al.* 2021.** *Nat Rev Microbiol* 19, 409-424. doi:10.1038/s41579-021-00573-0. **4. WHO, 2021,** https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ **5. Piccoli, L. *et al.* 2020.** *Cell* 183, 1024-1042 e1021. doi: 10.1016/j.cell.2020.09.037 **6. Bian, L. *et al.* 2021.** *Expert Rev Vaccines* 20, 365-373. doi: 10.1080/14760584.2021.1903879. **7. Garcia-Beltran, W.F. *et al.* 2021.** *Cell* 184(9):2372-2383.e9. doi: 10.1016/j.cell.2021.03.013. **8. Nasreen, S. *et al.* 2021.** *medRxiv*. doi: 10.1101/2021.06.28.21259420. **9. Brosh-Nissimov, T. *et al.* 2021.** *Clin Microbiol Infect*. Pre-Proof. doi: /10.1016/j.cmi.2021.06.036. **10. Kustin, T. *et al.* 2021.** *Nat Med*. doi: 10.1038/s41591-021-01413-7. **11. Buchrieser, J. *et al.* 2020.** EMBO J, e106267. doi:10.15252/embj.2020106267. **12. Asarnow, D. *et al.* 2021.** *Cell* 184, 3192-3204 e3116. doi: 10.1016/j.cell.2021.04.033.

Antibody responses to SARS-CoV-2 Spike variants

Our understanding of the antibody response to SARS-CoV-2 is continuously evolving, especially with the emergence of numerous variants of concern. InvivoGen offers a comprehensive family of tools to help determine the binding affinity and neutralization potential of anti-Spike antibodies from the serum of patients/vaccinees or recombinantly produced.

Anti-Spike detection assays

InvivoGen provides a collection of tools to study the binding of monoclonal antibodies (mAbs) and/or serum antibodies to SARS-CoV-2 Spike variants. They include a range of research- or clinically-relevant recombinant Anti-Spike mAbs. They have been functionally validated and are thus ideal benchmarks or controls for screening assays.

ELISA and LIPS

InvivoGen has developed RBD-Lucia proteins, a collection of luciferase-tagged Spike variant RBD proteins. They can be used to assess the binding affinity of anti-Spike antibodies using either ELISA or LIPS assays.

• Luciferase-based ELISA

RBD-Lucia proteins feature Lucia luciferase, a reporter that provides a larger dynamic range than the commonly used HRP detection. Using ELISA, in less than 2 hours (hands on time) these chimeric proteins can be used to compare the binding affinity of a given mAb to different Spike variants (*Fig. 1a*).

• LIPS

Luciferase immunoprecipitation system (LIPS) is a solution-phase assay, whereas ELISA is a solid-phase assay. It allows the detection of antibodies against both linear and conformational epitopes. Using LIPS, RBD-Lucia proteins allow the testing of anti-Spike Abs. Its main advantage over ELISA is the ability to assess the antibody response in the sera of recovered COVID-19 patients and/or vaccinees (*Fig. 1b*).

Endotoxin contamination of recombinant Spike proteins has been shown to create experimental bias[1]. As part of the rigorous quality control of our products, InvivoGen validates the absence of endotoxin contamination.

Flow cytometry (FACS)

InvivoGen offers a collection of pUNO1-Spike-dfur plasmids specifically designed for the screening of Anti-Spike mAbs using FACS-based binding assays upon transfection into a host cell (e.g. HEK293).
To facilitate maximum surface expression for FACS screening assays, the Spike furin cleavage site has been inactivated through the mutation of the crucial recognition residues (R683A and R685A).
HEK293 cells transfected with pUNO1-Spike-dfur plasmids encoding either the original or the P.1 Spike protein were used to readily assess by FACS the binding affinity of recombinant mAbs. As seen in *Fig. 2*, (*a*) Anti-CoV2RBD-ete-mIgG2a displays stronger binding affinity for the original Spike compared to the P.1 variant, whereas (*b*) Anti-CoV2RBD-bam-mIgG2a does not bind the same P.1 variant.

PRODUCTS	QTY	CAT. CODE
pUNO1-SpikeV'X'-dfur	20 µg	p1-spike-df-v'x'
Anti-CoV2RBD-cas-mIgG2a	100 µg	srbdc3-mab10
Anti-CoV2RBD-imd-mIgG2a	100 µg	srbdc4-mab10
Anti-CoV2RBD-bam-mIgG2a	100 µg	srbdc5-mab10
Anti-CoV2RBD-ete-mIgG2a	100 µg	srbdc6-mab10

'x' refers to the in-house number of the Spike variant (see table on the front page).
More info at: www.invivogen.com/sars2-spike-vectors

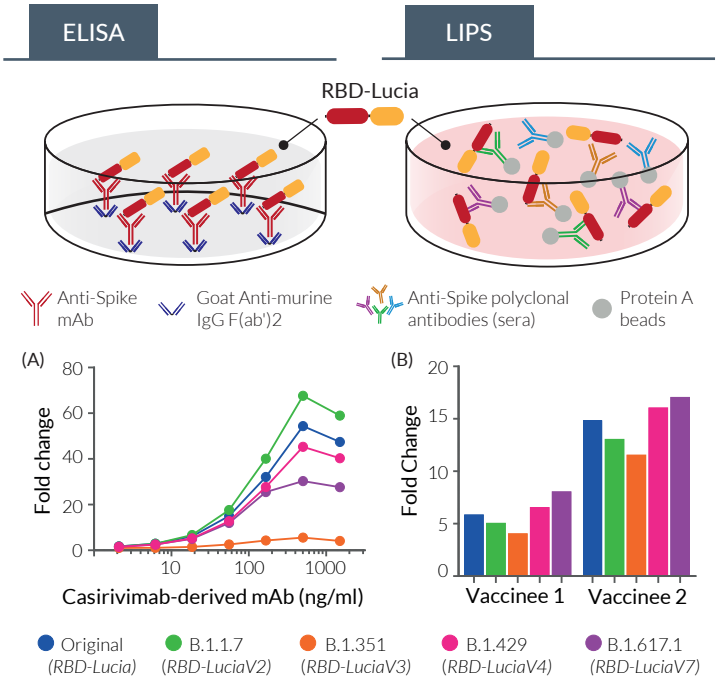


Fig 1: Binding affinity of Anti-Spike antibodies. (A) ELISA was performed by first adding an Anti-mIgG antibody, then Anti-CoV2RBD-cas-mIgG2a (Casirivimab-derived mAb), and lastly RBD-Lucia, RBD-LuciaV2, RBD-LuciaV3, RBD-LuciaV4 or RBD-LuciaV7 protein. **(B)** LIPS was performed by mixing Protein A beads, a vaccinee serum, and a RBD-Lucia protein (same as (A)). **(A+B)** Binding affinity was assessed by measuring Lucia activity using QUANTI-Luc™.

PRODUCTS	QTY	CAT. CODE
RBD-LuciaV'X'	20 µg	rbd-lucia-v'x'

'x' refers to the in-house number of the Spike variant (see table on the front page).
More info at: www.invivogen.com/sarscov2-lucia

1. Ouyang, W. et al. 2021. *Int. J. Mol. Sci.* 22(14), 7540. doi: 10.3390/ijms22147540.

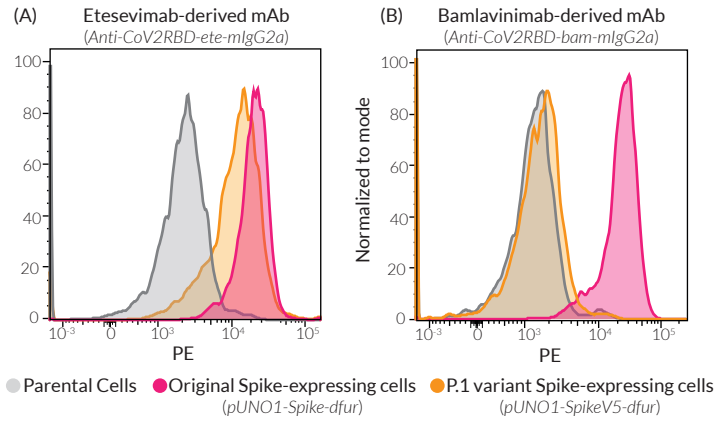


Fig 2: Detection of Spike variants by FACS. 293 parental (grey), 293-SARS2-S-dfur (original; pink), and 293-SARS2-S-dfur-V5 (P.1; orange) cells were incubated with either **(A)** Anti-CoV2RBD-ete-mIgG2a or **(B)** Anti-CoV2RBD-bam-mIgG2a for 1h at 4°C. Cells were then washed and incubated with a PE-conjugated goat anti-murine IgG for 1h at 4°C. Cell surface staining was analyzed by FACS.

We have many more SARS-CoV-2 products, so check out our website for the full collection!

https://www.invivogen.com/covid-19

Neutralization Assays

InvivoGen also offers an expanding collection of tools to study the neutralization potential of mAbs and/or serum antibodies from recovered COVID-19 patients or vaccinees against Spike variants.

Spike-pseudotyped lentiviral infection

Spike-pseudotyped lentiviral particles are commonly used as SARS-CoV-2 mimics to study viral entry into host cells without the need for a specialized biosafety laboratory (BSL-3).

InvivoGen provides a collection of pLV-Spike plasmids encoding various Spike variants. In combination with accessory and GFP reporter plasmids (*not provided*), these plasmids allow the generation of Spike-pseudotyped lentiviral particles. Infection of a permissive cell line expressing the SARS-CoV-2 host receptors (e.g. A549-hACE2-TMPRSS2 or HEK-Blue™ hACE2-TMPRSS2 cells) by pseudotyped particles is measured using GFP expression. This assay is ideal for studying viral entry and can be used to test the neutralization potential of antibodies as well as small molecule inhibitors.

Using lentiviral particles pseudotyped with the B.1.1.7 Spike, inhibition of infection was observed with all tested clinically-derived mAbs (*Fig 3a*), whereas infection by B.1.351 pseudotyped particles was blocked by the Imdevimab-derived mAb only (*Fig 3b*).

Spike-ACE2-dependent cell fusion

InvivoGen has developed an assay that mimics SARS-CoV-2 induced syncytia, a phenomenon observed in the lungs of severe COVID-19 patients. It is a simple, quantitative, and colorimetric assay to study Spike-ACE2-dependent cell fusion.

The readout of this assay relies on the transfer of the adaptor molecule, MyD88, from a 'donor cell line' to a permissive 'acceptor cell line' expressing an NF-κB-SEAP inducible reporter gene.

- 'Donor' cells: 293-hMyD88 cells co-expressing a Spike variant following transfection with a pUNO1-Spike plasmid.
- 'Acceptor' cells: HEK-Blue™ hACE2 or A549-Dual™ hACE2-TMPRSS2 cells.

Co-culture of 'donor' and 'acceptor' cells leads to Spike-ACE2-dependent fusion of their cell membranes. Overexpressed MyD88 in the 'donor' cells activates a signaling cascade that leads to NF-κB-dependent SEAP production by the acceptor cell. SEAP activity is assessable using the detection reagent, QUANTI Blue™ Solution.

The neutralizing ability of antibodies is determined by measuring the reduction of SEAP production. As seen in *Fig. 4*, the Anti-CoV2RBD-imd-mIgG2a blocks cell fusion of the original and P.1 variant, whereas, the Anti-CoV2RBD-cas-mIgG2a displays reduced inhibition against the same P.1 variant.

PRODUCTS	QTY	CAT. CODE
pUNO1-SpikeV'X'	20 µg	p1-spike-v'x'
293-hMyD88 Cells	3 -7 x 10^6 cells	293-hmyd
HEK-Blue™ hACE2 Cells	3 -7 x 10^6 cells	hkb-hace2
A549-Dual™ hACE2-TMPRSS2 Cells	3 -7 x 10^6 cells	a549d-cov2r

'x' refers to the in-house number of the Spike variant (see table on the front page).
More info at: www.invivogen.com/293-hmyd88

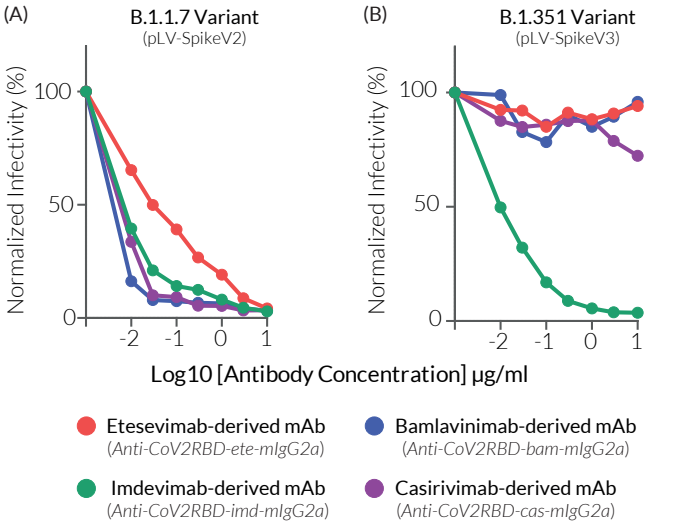


Fig 3: Spike-pseudotyped particle-based inhibition assay. A549-hACE2-TMPRSS2 cells were cultured in the presence of Anti-CoV2RBD-cas-mIgG2a, Anti-CoV2RBD-imd-mIgG2a, Anti-CoV2RBD-bam-mIgG2a or Anti-CoV2RBD-ete-mIgG2a antibodies. Either **(A)** B.1.1.7 (pLV-SpikeV2) or **(B)** B.1.351 (pLV-SpikeV3) Spike-pseudotyped GFP lentiviral particles were added to the cells. After 72h, the transduction efficiency (% infectivity) was evaluated by FACS.

PRODUCTS	QTY	CAT. CODE
pLV-SpikeV'X'	20 µg	plv-spike-v'x'
A549-hACE2-TMPRSS2 Cells	3 -7 x 10^6 cells	a549-hace2tpsa
HEK-Blue™ hACE2-TMPRSS2 Cells	3 -7 x 10^6 cells	hkb-hace2tpsa

'x' refers to the in-house number of the Spike variant (see table on the front page).
More info at: www.invivogen.com/spike-pseudotyping-vectors

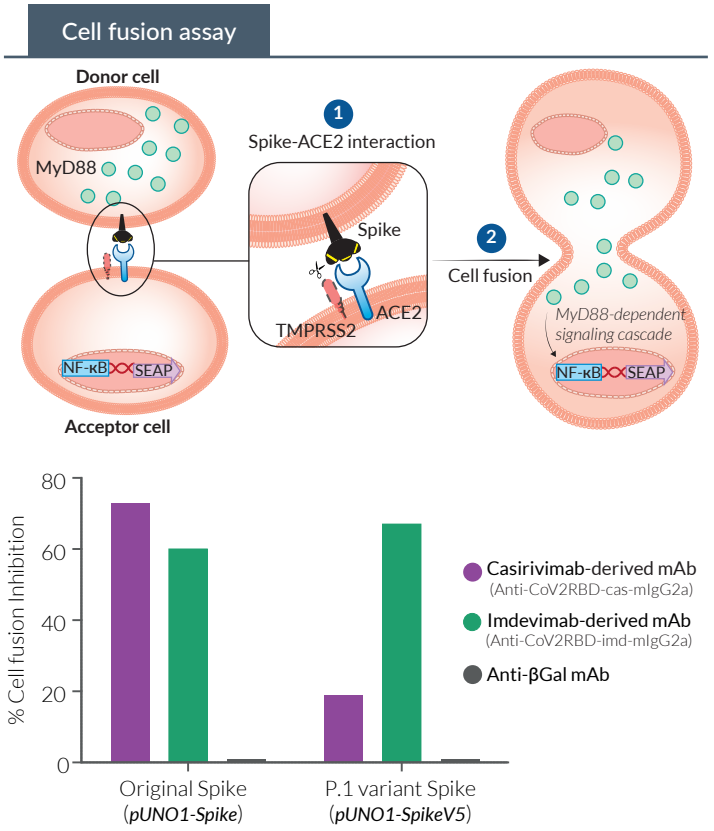


Fig 4: Cell fusion inhibition assay. 293-hMyD88 cells were transfected with either the pUNO1-Spike or pUNO1-SpikeV5 plasmid and then incubated with Anti-CoV2RBD-cas-mIgG2a, Anti-CoV2RBD-imd-mIgG2a, or Anti-βGal antibodies. These cells were then co-cultured with HEK-Blue™ hACE2 cells. After overnight incubation, cell fusion was assessed by measuring the activity of SEAP in the supernatant using QUANTI-Blue™ Solution.

The new way to detect Mycoplasma

With over 40 years of experience in developing mycoplasma solutions for the scientific community, InvivoGen now offers MycoStrip™, a simple strip-based test that requires no special lab equipment for the detection of mycoplasma in cell culture. Importantly, with results in less than an hour, MycoStrip™ allows you to swiftly combat any contamination.

• MycoStrip™ NEW

Forget other laborious detection methods! MycoStrip™ is an easy-to-use and highly sensitive method to rapidly detect mycoplasma contamination in cell cultures. Detection by MycoStrip™ is based on isothermal PCR. Simply prepare your sample and add our proprietary Reaction Mix to target and amplify the 16S rRNA gene of the most commonly found mycoplasma species in cell culture. Results are clearly visualized as a band on a lateral flow detection strip within 5 minutes.

Key Features

- Simple: Only a heat block is required
- Rapid: Results within 2 - 5 minutes
- Easy interpretation: 1 or 2 bands on the strip
- Minimal hands-on-time: <15 minutes
- Total test duration: <1 hour
- Highly sensitive: Detects as low as 1 x 10^2 CFU/ml
- Specific: No cross-reactivity
- Stable: The kit can be kept 1 year at -20°C

Mycoplasma Detection with MycoStrip™

MycoStrip™ has been specifically designed to detect the Mycoplasma and Acholeplasma species that most commonly contaminate cell cultures. These include the six species (see below) that account for 95% of all contaminations. Importantly, there is no cross-reactivity with any other tested bacterial, fungal, or mammalian DNA when using MycoStrip™.

MYCOPLASMA:
M. orale
M. hyorhinis
M. arginini
M. fermentans
M. hominis

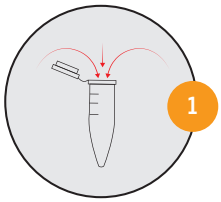
ACHOLEPLASMA:
A. laidlawii

PRODUCT	DESCRIPTION	QTY	CAT. CODE
MycoStrip™	Mycoplasma contamination detection kit (strips)	10 tests 20 tests 50 tests	rep-mys-10 rep-mys-20 rep-mys-50

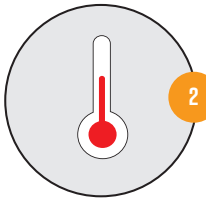
www.invivogen.com/mycostrip

Detection with MycoStrip™

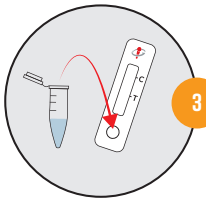
DETECT MYCOPLASMA IN 4 EASY STEPS



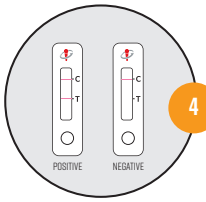
1 PREPARE YOUR SAMPLE (~5 MINS)



2 INCUBATE AT 65°C FOR 40 MINUTES



3 STOP THE REACTION AND LOAD THE CASSETTE



4 READ YOUR RESULTS WITHIN 2-5 MINUTES

WHAT IF MY TEST IS POSITIVE?

Don't stress! Your culture is easily treatable with InvivoGen's anti-microbial product range.

Treat your culture and eradicate the contamination using our two highly-cited and fast-acting mycoplasma removal reagents, **Plasmocin™** or **Plasmocure™**. Upon completion of the treatment (~2 weeks), simply re-test using MycoStrip™ comparing your newly treated culture with your previous sample.

For more information visit: www.invivogen.com/mycoplasma-elimination

InvivoGen

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