InvivoGen insight

SUMMARY :

✎ REVIEW

Interferons λs: guardians of the front-lines

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- HEK-Blue™ IFN-λ cells

Interferon-λ Antibodies
- Anti-hIL-28a-IgG (hIFN-λ2)
- Anti-hIL-28b-IgG (hIFN-λ3)
- Anti-hIL-29-IgG (hIFN-λ1)

JAK/STAT Signaling Inhibitors
- CP-690550
- CYT387
- Ruxolitinib

Recombinant Type I Interferons
- Recombinant human interferon-αs

Mycoplasma Detection & Elimination

InvivoGen

Interferon λs: guardians of the front-lines

Interferons (IFNs) are key cytokines of the innate immune system known for their antiviral and immuno-modulatory properties. Three types of IFNs have been described: type I IFNs which are mainly comprised of IFN-αs and IFN-β, type II IFN or IFN-γ, and the most recently discovered type III IFNs or IFN-λs[1,2]. Although IFN-α/β and IFN-λs share many overlapping functions, a unique role at the mucosal barrier sites has emerged for IFN-λs.

In humans, the IFN-λ family consists of 4 proteins, IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4[2]. IFN-λ1-3 exhibit high amino acid sequence homologies, whereas IFN-λ4 is more divergent. In mice, only IFN-λ2 and IFN-λ3 are functional[2]. IFN-λs are induced after viral infection by pattern recognition receptors (PRRs) that sense viral nucleic acids, including members of the Toll-like receptor (TLR) and RIG-I-like receptor (RLR) families[3]. These PRRs signal through adaptor proteins such as MAVS to activate interferon regulatory factors (IRFs) and NF-κB leading to IFN-λ expression. In contrast, PRR-induction of type I IFNs is mainly IRF3-dependent[2-4].

Many cell types can produce IFN-λs but the most potent producers are dendritic cells and epithelial cells (ECs) in barrier organs such as skin, lungs, liver and the gastro-intestinal (GI) tract[2]. The preferential production of IFN-λs over IFN-α/β in ECs has been linked to the abundance of peroxisomes in these cells and the shift of MAVS from mitochondria to peroxisomes, promoting IRF1-induced expression of type III IFNs[5].

IFN-λs bind to IL-28R, a heterodimeric receptor comprised of IL-10Rβ (shared by the IL-10 cytokine family) and IFNRL1 (or IL-28RA, specific for IFN-λs). In contrast to the broad expression of IL-10Rβ, IFNRL1 expression is restricted to ECs and immune cell subsets. Upon receptor binding, IFN-λs induce an antiviral response, very similar to the one triggered by IFN-α/β. Both IFN types engage a common JAK–STAT pathway leading to the formation of the ISGF3 transcriptional complex and the expression of

hundreds of IFN-stimulated genes (ISGs) that mediate a variety of activities[2,3].

The antiviral activity of IFN-λs is prevalent in lungs, GI tract, and liver, consistent with the predominant expression of IL-28R in epithelial tissues[3]. Upon virus infection, both type I and type III IFNs trigger the expression of antiviral ISGs, but only IFN-αs induce pro-inflammatory ISGs. Moreover, IFN-λs activate a lower but prolonged expression of ISGs compared to IFN-αs[6]. The current view is that ECs exposed to viral stimuli produce IFN-λs clearing the infection and preventing its spread to neighboring cells. In the case of high viral burden and/or escape from the control of IFN-λs, type I IFNs are produced to enhance the antiviral response and promote inflammatory responses in the epithelium and beneath[7]. The localized and specific antiviral response induced by type III IFNs ensures host fitness and reduced risks of diseases caused by excessive type I IFN activity.

IFN-λ activity varies among the different subtypes depending on their affinity for IL-28R, which is generally low, and the presence of single nucleotide polymorphisms (SNPs)[3,6]. A number of SNPs in the IFN-λ and IL-28R genes have been identified and associated with both improved and worsened clinical outcomes, particularly in the context of viral hepatitis[8]. For example, two SNPs linked to ethnic ancestry have been reported in the IFN-λ4 gene: one resulting in a pseudogene and the other generating a functional allele which confers a high risk of hepatitis-C chronicity[9]. The role of SNP variation on IFN-λ signaling may affect more infectious diseases than viral hepatitis and is thus thoroughly investigated.

Beyond their role in the antiviral response at mucosal barrier sites, IFN-λs have been shown to participate in the antibacterial response, the adaptive response to viral infection, autoimmunity and anti-tumor responses[3,10]. New data are emerging, highlighting the non-redundant functions of IFN-λs and their therapeutic potential for treating infectious diseases with minimum systemic toxicity.

Epithelial cells
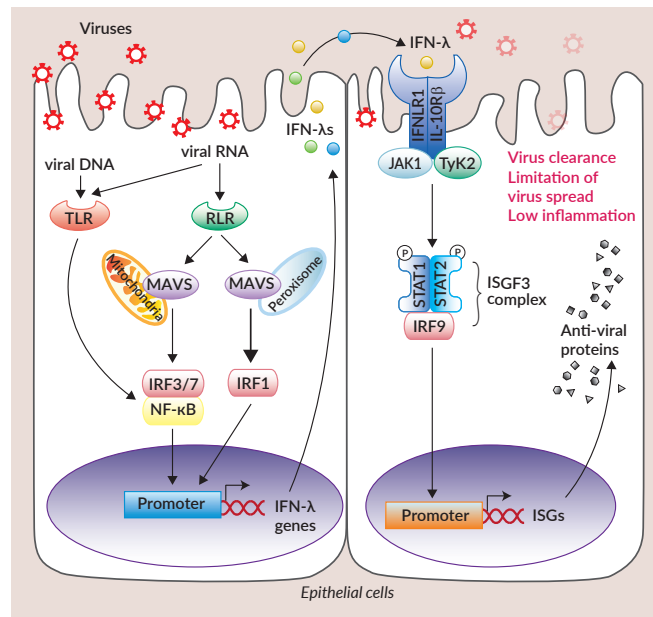
1. Pestka S., 2007. The Interferons: 50 years after their discovery there is much more to learn. J. Biol. Chem. 282: 20047. 2. Kotenko S.V. and Durbin J.E., 2017. Contribution of type III interferons to antiviral immunity: location, location, location. J. Biol. Chem. 292: 7295. 3. Lazear H.M. et al., 2015. Interferon-λ: Immune Functions at Barrier Surfaces and Beyond. Immunity 43: 15. 4. Durbin R.K. et al., 2013. Interferon induction and function at the mucosal surface. Immunol. Rev. 255: 25. 5. Odendall C. et al., 2014. Diverse intracellular pathogens activate Type III interferon expression from peroxisomes. Nat. Immunol. 15:717. 6. Bolen C.R. et al., 2014. Dynamic expression profiling of type I and type III interferon-stimulated hepatocytes reveals a stable hierarchy of gene expression. Hepatology. 59: 1262. 7. Andreakos E. et al., 2017. Interferon-λs: front-line guardians of immunity and homeostasis in the respiratory tract. Front. Immunol. 8: 1232. 8. Syedbasha M. and Egli A., 2017. Interferon Lambda: Modulating Immunity in Infectious Diseases. Frontiers in Immunology 8.: 119. 9. Obajemu A.A. et al., 2017. IFN-λ4 Attenuates Antiviral Responses by Enhancing Negative Regulation of IFN Signaling. J. Immunol. 199: 3808. 10. Zanoni I. et al., 2017. Interferon (IFN)-λ Takes the Helm: Immunomodulatory Roles of Type III IFNs. Front. Immunol. 8: 1661.

Interferon Lambda Reporter Cell Line

HEK-Blue™ IFN-λ Cells   NEW

- **Specific:** detect human and mouse IFN-λs only
- **Highly sensitive:** similar to an ELISA
- **Convenient:** colorimetric SEAP read-out assay

HEK-Blue™ IFN-λ cells are HEK293-derived reporter cells engineered to specifically respond to type III IFNs. They stably express the human IFNLR1 and IL10Rβ genes, coding for the IFN-λ receptor, and the human STAT2 and IRF9 genes. They are knocked out for the hIFNAR2 and hIFNGR1 genes encoding subunits of IFN-α/β and IFN-γ receptors. These cells also carry an ISG-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Stimulation of HEK-Blue™ IFN-λ cells with recombinant human or murine IFN-λ or with supernatants of IFN-λ-producing cells, such as A549 cells activated with RNA or THP-1 cells activated with DNA or cyclic dinucleotides (CDNs), leads to an ISG response and the production of SEAP. Levels of SEAP can be easily determined with QUANTI-Blue™, a SEAP colorimetric detection reagent.
HEK-Blue™ IFN-λ cells are resistant to Blasticidin, Puromycin and Zeocin™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IFN-λ cells	3-7 x 10^6 cells	hkb-ifnl
Quanti-Blue™ Solution	5 ml	rep-qbs
Blasticidin	5 x 1 ml	ant-bl-05
Puromycin	10 x 1 ml	ant-pr-1
Zeocin™	5 x 1 ml	ant-zn-05
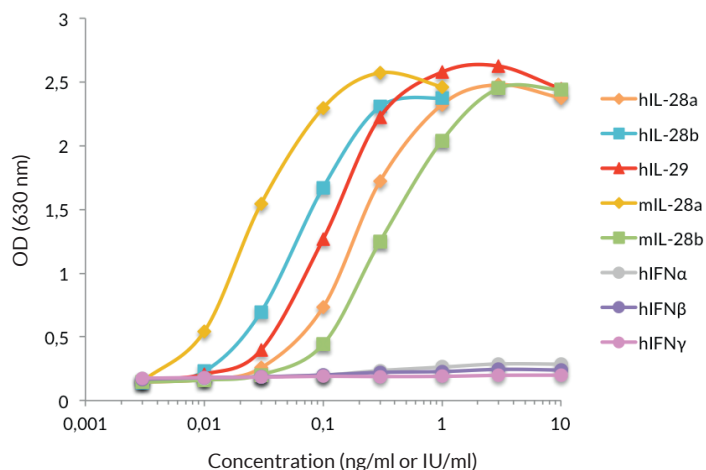
Cytokine	EC_{50} (ng/ml)
hIL28a (hIFN-λ2)	0.19
hIL28b (hIFN-λ3)	0.06
hIL29 (hIFN-λ1)	0.10
mIL28a (mIFN-λ2)	0.02
mIL28b (mIFN-λ3)	0.37
hIFN-α	N/A
hIFN-β	N/A
hIFN-γ	N/A

Response of HEK-Blue™ IFN-λ cells to type I, II and III IFNs: HEK-Blue™ IFN-λ cells were incubated with increasing concentrations of recombinant human or mouse IFN-λ (ng/ml) or human IFN-α, IFN-β or IFN-γ (IU/ml). After 24h incubation, ISG activation was assessed by measuring SEAP levels in the supernatant using Quanti-Blue™. EC_{50} is indicated for each cytokine (N/A: non applicable).

🌐 **www.invivogen.com/cytokine-reporter-cells**

Interferon Lambda Antibodies

- **Anti-hIL-28a-IgG** (hIFN-λ2)
- **Anti-hIL-28b-IgG** (hIFN-λ3)
- **Anti-hIL-29-IgG** (hIFN-λ1)

InvivoGen offers mouse monoclonal antibodies targeting the three major human IFN-λ isoforms. They have been selected for their ability to efficiently neutralize the biological activity of IFN-λs. These antibodies are produced in hybridomas and purified by affinity chromatography. Their neutralizing activity is validated using the HEK-Blue™ IFN-α/β cell line which detects type I and type III IFNs. The use of each antibody in three parallel assays allows the user to determine the major IFN-λ isoform present in a cell supernatant. InvivoGen's IFN-λ antibodies do not cross-react with mouse IFN-λs.

PRODUCT	QUANTITY	CAT. CODE
Anti-hIL28a-IgG	3 x 100 µg	mabg-hil28a-3
Anti-hIL28b-IgG	3 x 100 µg	mabg-hil28b-3
Anti-hIL29-IgG	3 x 100 µg	mabg-hil29-3
HEK-Blue™ IFN-α/β cells	3-7 x 10^6 cells	hkb-ifnab

Antibody	IC_{50} (ng/ml)
Anti-hIL28a IgG	6.6
Anti-hIL28b IgG	20.4
Anti-hIL29 IgG	86.1
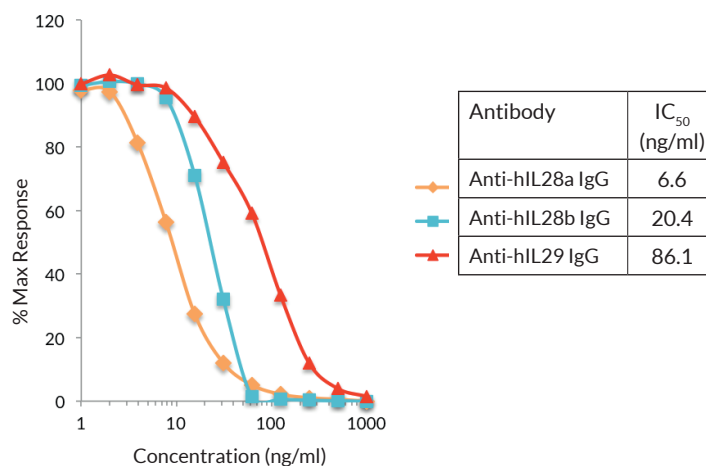
Neutralizing activity of interferon lambda antibodies: HEK-Blue™ IFN-α/β cells were incubated with 10 ng/ml recombinant hIL-28a, hIL28b, hIL-29 and increasing concentrations of their cognate antibody. After 24h incubation, recombinant IFN-λ induced ISG activation was assessed by measuring SEAP levels in the supernatant using Quanti-Blue™. Percentages of maximal response (no antibody) and IC_{50} for 10 ng/ml of each cytokine is shown.

🌐 **www.invivogen.com/antibodies**

JAK/STAT Signaling Inhibitors

- **CP-690550** - Pan-JAK inhibitor
- **CYT387** - JAK1/2 and TYK2 inhibitor
- **Ruxolitinib** - Pan-JAK inhibitor

> - High quality: purity > 95%, sterile-filtered, absence of TLR2/TLR4 activation confirmed
> - inhibitory activity validated: using cellular assays

InvivoGen offers a selection of Janus kinase (JAK) inhibitors known to interfere with IFN signaling. Indeed, extracellular signals from IFNs are transduced by JAK and signal transducer and activator of transcription (STAT) signaling pathway, ultimately leading to the transcription of IFN-stimulated genes (ISGs). CP-690550, CYT387 and ruxolitinib display different affinities for the four JAK members, JAK1/2/3 and TYK2[1], and therefore represent valuable tools to study the regulation of IFN signaling. These three inhibitors are functionally validated using recombinant IFNs in human and murine cellular assays. They appear to be as effective in blocking the IFN-λ (IL-29) than the IFN-α/β signaling pathway.

1. Roskoki R. Jr, 2016. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. Pharmacol. Res. 111:784-803.

🌐 **www.invivogen.com/jakstat-inhibitors**
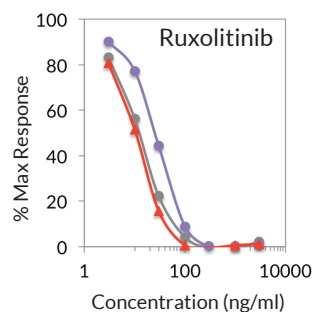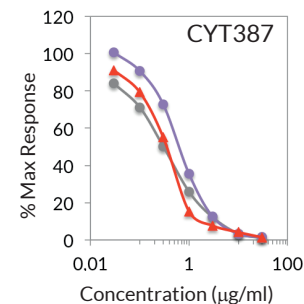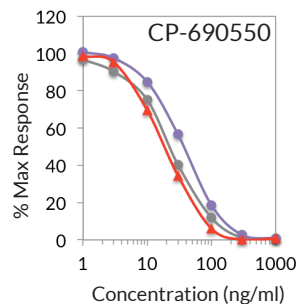
Effect of JAK/STAT inhibitors on HEK-Blue™ IFN-α/β cell response to type I and type III IFNs: HEK-Blue™ IFN-α/β cells were incubated with 3 U/ml hIFN-α2b (**grey**), 1 U/ml hIFN-β1 (**purple**) or 10 ng/ml hIL-29 (hIFN-λ1) (**red**) and increasing concentrations of JAK inhibitors. After 24h incubation, IFN-induced ISG activation was assessed by measuring SEAP levels in the supernatant using Quanti-Blue™. Percentages of maximal response (no inhibitor) for each cytokine are shown.

PRODUCT	QUANTITY	CAT. CODE
CP-690550 (Tofacitinib)	5 mg	tlrl-cp69
CYT387 (Momelotinib)	10 mg	inh-cy87
Ruxolitinib (INC424)	5 mg	tlrl-rux

Recombinant Type I Interferons

Recombinant human interferon-αs `NEW`

> - Mammalian source: produced in CHO or HEK293 cells
> - Functionally tested: using THP1-Dual™ cells
> - High quality: purity > 95%, endotoxin < 1 EU/µg

Type I interferons (IFNs) include the IFN-α family, which comprises 12 distinct proteins. All IFN-α subtypes bind to a unique heterodimeric receptor (IFNAR1/R2) and trigger the JAK1/TYK2/ISGF3 pathway inducing the expression of various interferon stimulated genes (ISGs)[1]. The patterns of ISG expression depend on the binding affinity of the different IFN-αs to their receptor. IFN-αs with low affinity for IFNAR1/R2 signal strictly through ISGF3 and induce "robust" ISGs with anti-viral functions. Conversely, IFN-αs with high affinity for the receptor signal through ISGF3 and other factors activating "tunable" ISGs with anti-proliferative and immuno-modulatory functions[2]. IFN-α8, -α10 and -α14 have been identified as the most potent inducers of ISGs, while IFN-α1 appears to be the weakest[3].
InvivoGen offers all twelve IFN-αs as recombinant proteins produced in mammalian cells and thoroughly validated using cell-based assays. They are provided individually or as a set, to meet your research needs.

1. **Hoffmann H-H.** *et al.*, **2015.** Interferons and viruses: an evolutionary arms race of molecular interactions. Trends Immunol. 36:124. **2. Schreiber G. & Piehler J., 2015.** The molecular basis for functional plasticity in type I interferon signaling. Trends Immunol. 36:139. **3. Kurunganti S.** *et al.*, **2014.** Production and characterization of thirteen human type-I interferon-α subtypes. Protein Expr. Purif. 103:75.
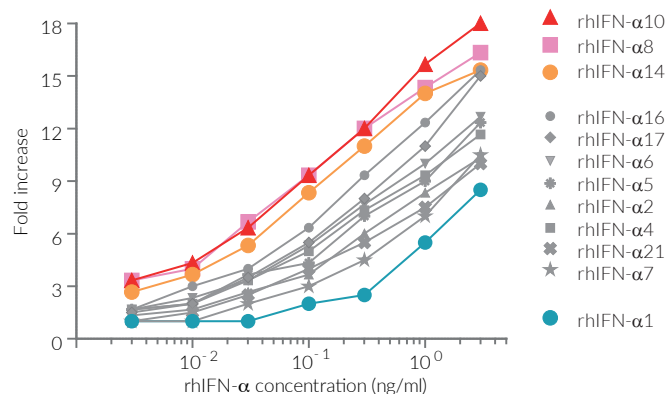
Response of THP1-Dual™ cells to recombinant human IFN-αs: THP1-Dual™ cells were incubated with increasing concentrations of each recombinant human IFN-α subtype (ng/ml). After 24h incubation, ISG activation was assessed by measuring Lucia luciferase activity in the supernatant using Quanti-Luc™.

PRODUCT	QUANTITY	CAT. CODE
Recombinant hIFN-α"n"	1 µg	rcyc-hifna"n"
Recombinant hIFN-α set	12 x 1 µg	rcyck-hifna
THP1-Dual™ cells	3-7 x 10^6 cells	thpd-nfis

"n" refers to the number of the IFN-α subtype, e.g. rhIFN-α2.

Recombinant human IFN-αs comprise IFN-α1 (D), -α2 (2b), -α4 (4a, M1), -α5 (G), -α6 (K), -α7 (J1), -α8 (B2), -α10 (C), -α14 (H2), -α16 (WA), -α17 (I) and-α21 (F).

🌐 **www.invivogen.com/human-ifna**

Mycoplasma Detection and Elimination

Mycoplasma contamination remains a major problem in cell culture, affecting the validity of experimental results as well as the quality and safety of cell-based biopharmaceuticals. Because of their small size (≤ 0.8 μm) and lack of a rigid cell wall, mycoplasmas are undetectable by visual inspection, pass through standard filtration and are resistant to a great number of antibiotics[1].

Mycoplasmas compete with host cells for nutrients and biochemical precursors and thus can alter many cell functions, such as cell metabolism and cell growth, ultimately leading to cell death. Upon adhesion or fusion interactions with the host cell membrane, they can cause further damage to the cell including interference with signaling cascades and cytokine production[2]. Such detrimental effects can strongly impact scientific results and invalidate the findings of a study, especially when the study involves immune cells which express Toll-like receptor 2 (TLR2), a pattern recognition receptor that recognizes mycoplasma lipoproteins[3].

Thus, many reasons support the need to establish routine detection of mycoplasma contamination in cell cultures and the use of specific antibiotics to save valuable cell lines. InvivoGen offers highly referenced solutions for the protection of your cell lines.

1. Drexler H.G and Uphoff C.C, 2002. Mycoplasma contamination of cell cultures: incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 39:75. 2. Rottem S. 2003. Interaction of Mycoplasmas with host cells. Physiol Rev. 83417. 3. Zakharova E. *et al.*, 2010. Mycoplasma suppression of THP-1 Cell TLR responses is corrected with antibiotics. PLoS One. 5(3):e9900.

PlasmoTest™

Detection of Mycoplasma Contamination in Cell Cultures

- **Reliable:** No false positive
- **Rapid:** Hands-on time <1 hour. Results after overnight incubation
- **Simple:** Colorimetric detection in cell culture supernatants

PlasmoTest™ is a cell-based mycoplasma detection assay that relies on the recognition of mycoplasmas by TLR2. It utilizes HEK-Blue™-2 sensor cells which produce the SEAP reporter protein upon TLR2 triggering. Addition of test samples to these cells provides colorimetric results with sensitivity similar to luminescence-based biochemical assays. PlasmoTest™ is provided as a kit containing the sensor cells, SEAP detection culture medium, and positive and negative controls.

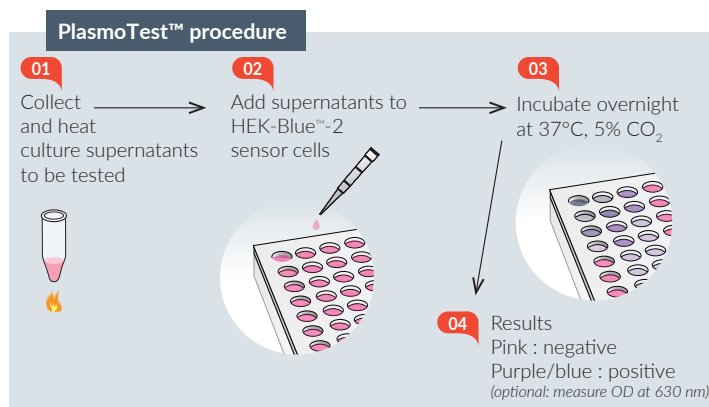## Plasmocin™ & Plasmocure™

Elimination of Mycoplasma Contamination in Cell Cultures

- **Potent:** Eradicate all mycoplasmas in 2 weeks
- **Safe:** No cell alteration
- **Easy to use:** Simply add to cell culture medium

Plasmocin™ is a widely used mycoplasma removal agent and is highly effective against most mycoplasma strains. Plasmocure™ is a second-line anti-mycoplasma reagent that potently eradicates Plasmocin™-resistant mycoplasmas. With Plasmocin™ and Plasmocure™, elimination of mycoplasma contamination in cell cultures is guaranteed.

Major impacts of mycoplasma contamination on cell functions
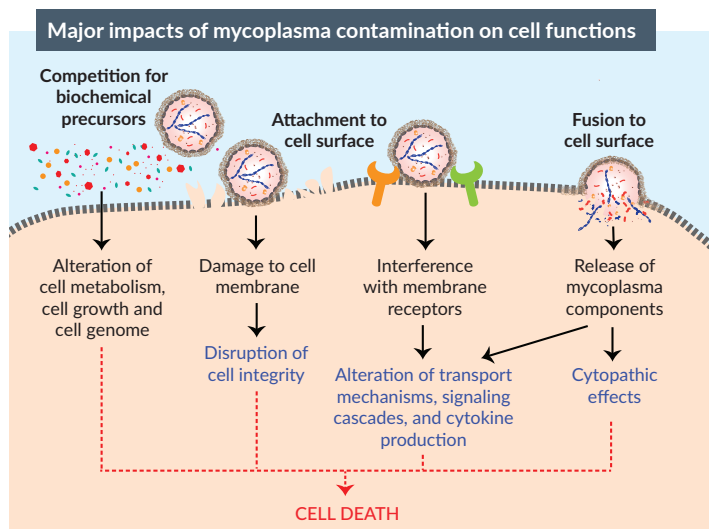
TOP 5 REASONS TO TEST

1. 20 to 35% worldwide contamination, including cell banks
2. Serious impact on data reliability and reproducibility
3. Loss of cell lines
4. Financial impact
5. Mycoplasma testing required by most journals

PlasmoTest™ procedure

01 Collect and heat culture supernatants to be tested

02 Add supernatants to HEK-Blue™-2 sensor cells

03 Incubate overnight at 37°C, 5% CO_2

04 Results
Pink : negative
Purple/blue : positive
(optional: measure OD at 630 nm)

PRODUCT	QUANTITY	CAT. CODE
PlasmoTest™	1 kit (250 tests)	rep-pt1
PlasmoTest™ controls	200 tests	pt-ctr2
PlasmoTest™ refills	500 tests	rep-ptrk
Plasmocin™	25 mg (1 ml)	ant-mpt-1
Plasmocure™	100 mg (1 ml)	ant-pc

www.invivogen.com/mycoplasma