December 2006

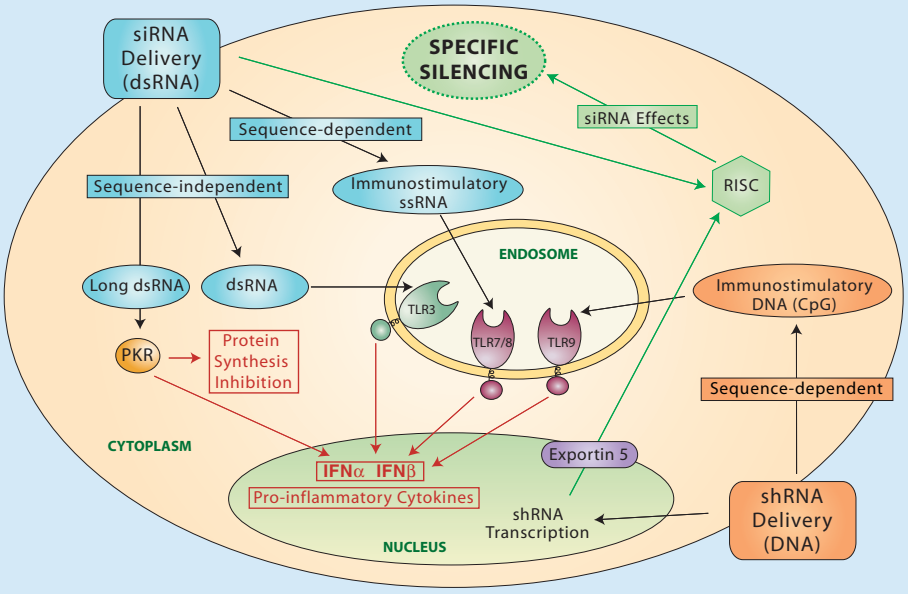
InvivoGen Insight

In this issue, you will find the solution to your mycoplasma problem. InvivoGen provides PlasmoTest™, a simple and reliable mycoplasma detection system, and Plasmocure™, a new mycoplasma removal agent. You will also find precomplexed psiRNAs that express shRNAs targeting a selection of genes involved in the TLR pathway. These formulations display higher efficiency than traditional psiRNA complexes. Since certain siRNAs and shRNAs can induce an IFN response, InvivoGen has designed a set of qRT-PCR primers to detect genes involved in this response. Lastly, we introduce two new promising geldanamycin analogues, 17-AAGH2 and 17-DMAGH2, the hydroquinone and more active forms of 17-AAG and 17-DMAG.

Inside this issue:

InvivoGen
3950-A Sorrento Valley Blvd
San Diego, CA 92121 USA

RNA interference: Induction of the IFN Response

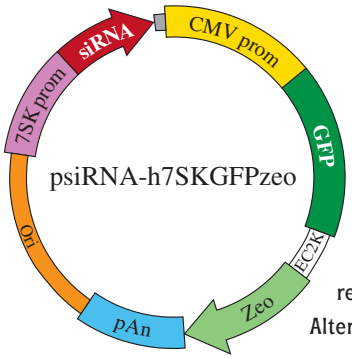


RNA interference (RNAi) has become a powerful tool to uncover gene function and is evolving as a new therapeutic modality. The mediators of RNAi are double stranded (ds) small interfering RNAs (siRNAs) cellularly delivered as synthetic duplexes or as short hairpin RNAs (shRNAs) by plasmids or viral vectors. siRNAs are incorporated into the RNA interference specificity complex (RISC) leading to the cleavage and degradation of the target mRNA.

A growing concern when inducing RNAi whether using synthetic siRNAs or shRNAs is the activation of an immune response. Recent studies report that siRNAs can be potent inducers of interferons (IFNs) and inflammatory cytokines both *in vivo* and *in vitro*[1-3] raising questions about the specificity of RNAi. Much of the IFN response is caused by the activation of the dsRNA-dependent protein kinase R (PKR) leading to a global inhibition of protein synthesis. Yet siRNAs that are shorter than 30 bp can evade PKR activation. Toll-like receptor (TLR) 3 is another receptor for dsRNA and seems to be involved in siRNA-induced IFN response[4]. However, this recognition appears to be cell-specific as not all immune cells express TLR3. Activation of immune cells by siRNAs is also sequence dependent and sense or antisense strands separately can induce cytokine production as efficiently as ds siRNAs[1-3]. Thus, other TLRs may play a role in the stimulatory effect of siRNAs. Recent data indicate that TLR7 and/or TLR8 mediate the recognition of ds and single strand (ss) siRNAs in a sequence-dependent manner[1-3]. U-rich and GU-rich siRNAs seem to be preferentially recognized but some siRNAs were found to be stimulatory independently of their GU content suggesting the existence of specific sequences recognized by TLR7/8. TLR7 and TLR8 are endosomal receptors and require the compartmentalization of siRNAs in endosomes to be activated. Indeed, lipid-delivered siRNAs that localize in the endosome can be immunostimulatory in contrast to siRNAs delivered in the cytoplasm by electroporation[3].

The immunostimulatory "side effects" of siRNAs must be taken in account when inducing RNAi. InvivoGen is introducing a set of primers designed to detect an IFN-response by real-time quantitative PCR. In order to limit this IFN response and other stimulatory effects, InvivoGen is updating the siRNA Wizard, its free siRNA-design software, to exclude sequences that are potentially immunostimulatory (sirnawizard.com). Furthermore, as shRNAs appear to be less stimulatory than synthetic siRNAs, the use of pCpG-siRNA, a CpG-free plasmid designed for the production of shRNAs that does not activate TLR9, may further limit the induction of an siRNA-induced immune response.

1. Hornung V. et al., 2005. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat Med. 11(3):263-70.
2. Judge AD. et al., 2005. Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Nat Biotechnol. 23(4):457-62.
3. Sioud M., 2005. Induction of inflammatory cytokines and interferon responses by double-stranded and single-stranded siRNAs is sequence-dependent and requires endosomal localization. J Mol Biol. 348(5):1079-90.
4. Kariko K. et al., 2004. Exogenous siRNA mediates sequence-independent gene suppression by signaling through toll-like receptor 3. Cells Tissues Organs. 177(3):132-8.

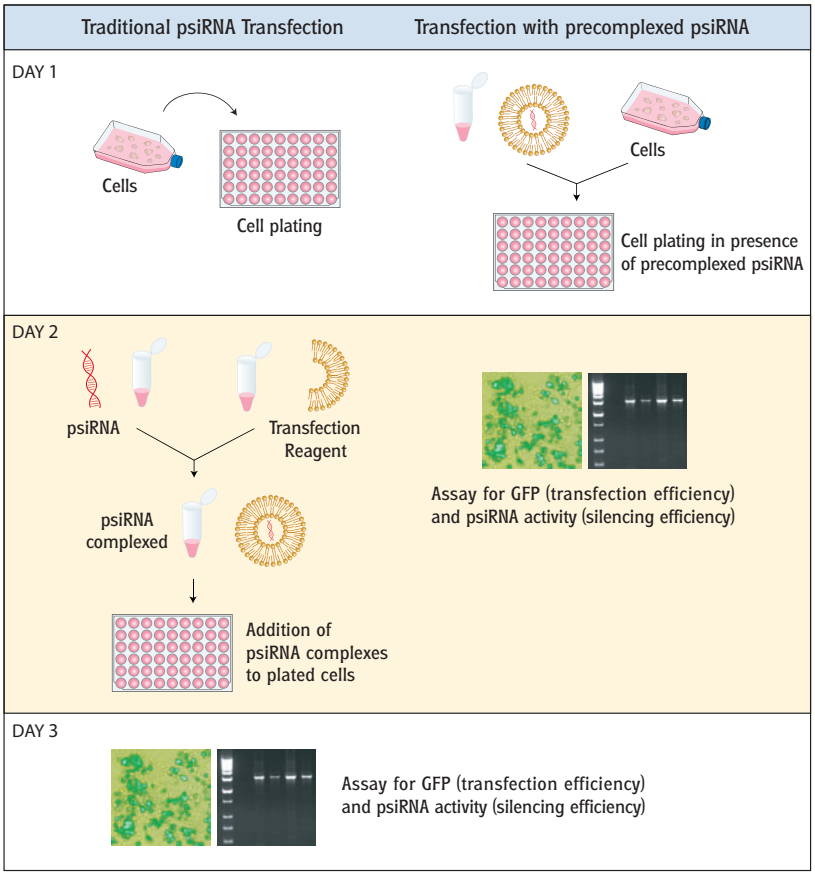


Precomplexed psiRNAs NEW

InvivoGen provides a large collection of psiRNA plasmids expressing functional shRNAs. To facilitate transfection of a wide variety of cells, InvivoGen offers a selection of precomplexed psiRNAs that target human or reporter genes. These formulations allow for faster reading, higher efficiency and more reproducible results than extemporaneous preparation of psiRNA complexes using conventional transfection reagents. Since psiRNA plasmids express a GFP reporter system, transfection efficency is easy to assess. Alternatively, transfected cells can be sorted out by FACS or stable clones selected using Zeocin™.

Precomplexed psiRNAs are generated by complexing endotoxin-free psiRNA plasmid DNA with an optimized formulation of the transfection reagent LyoVec™. These ready-to-use complexed psiRNAs are sold as a lyophilized powder (15 µg of DNA).

Product	Species	Catalog Code
TLR1	Human	lypsi-htlr1
TLR2	Human	lypsi-htlr2
TLR3	Human	lypsi-htlr3
TLR4	Human	lypsi-htlr4
TLR5	Human	lypsi-htlr5
TLR6	Human	lypsi-htlr6
TLR7	Human	lypsi-htlr7
TLR8	Human	lypsi-htlr8
TLR9	Human	lypsi-htlr9
IPS1	Human	lypsi-hips1
Mda5	Human	lypsi-hmda5
RIG-I	Human	lypsi-hrigi
MyD88	Human	lypsi-hmyd88
TICAM1	Human	lypsi-hticam1
IRAK1	Human	lypsi-hirak1
IRAK4	Human	lypsi-hirak4
TRAF6	Human	lypsi-htraf6
LucGL3	-	lypsi-hlucgl3



IFNr qRT-Primers - Detection of the IFN Response NEW

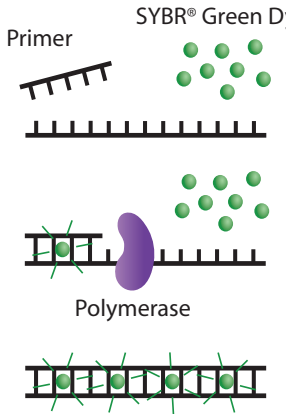
To determine whether a given siRNA or shRNA is immunostimulatory, InvivoGen has developed a set of primers that detect the expression of human genes involved in the IFN response by real-time quantitative PCR using the SYBR® Green detection.

The IFNr qRT-Primers are a set of primer pairs designed to measure the mRNA expression of 5 genes involved in the IFN response: IFNβ, OAS1, MX1 (also known as MxA), G1P2 (also known as ISG15), IFIT1 (also known as ISG56), and the housekeeping gene GAPDH as a control.

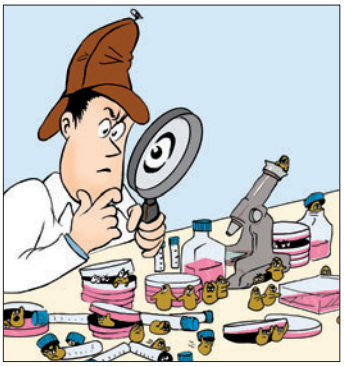
The IFNr qRT-Primers contain 10 µM of each primer. The 5' sense primer and the 3' antisense primer are provided in separate vials. Products are lyophilized and shipped at room temperature.

- The IFNr qRT-Primers provide highly specific and sensitive results in real-time quantitative PCR.
- Each IFNr qRT-Primer Pair is carefully designed and tested.
- The size of the amplified fragments varies from 60 to 200 bp.
- The IFNr qRT-Primers allow to perform 100 x 50 µl reactions (in a 96-well plate) or 250 x 20 µl reactions (in a 384-well plate).

Product	Quantity	Catalog Code
IFNr qRT-Primers	1 kit	rts-hinfr



During extension step of PCR, IFNr qRT-Primers anneal to template and SYBR® Green dye binds to double stranded DNA emitting fluorescent light.



Mycoplasma Detection and Elimination

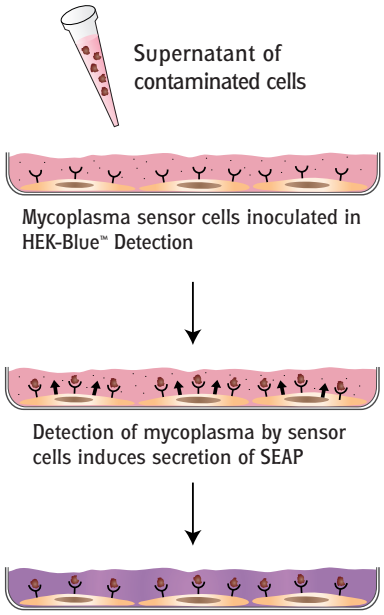
Mycoplasma contamination remains a significant problem to the culture of mammalian cells. Mycoplasmas are the smallest and simplest self-replicating organisms. They cannot be detected by visual inspection and they do not cause consistent perceptible changes in a cell culture, in contrast to other microbial contaminants. Mycoplasmas can cause disastrous effects on eukaryotic cells as they can alter every cellular parameter from proliferation to virus susceptibility and production.

PlasmoTest™ - Mycoplasma Detection Right in your Incubator

The only way to confirm mycoplasma contamination is by routine testing using special techniques. InvivoGen has developed PlasmoTest™, a simple, rapid and reliable cell-based colorimetric assay for the detection of mycoplasma in cell cultures. PlasmoTest™ is the first mycoplasma detection kit that uses engineered cells and therefore can be easily established as a routine procedure in the lab.

PlasmoTest™ features two major constituents: the Mycoplasma sensor cells and the HEK-Blue™ Detection medium. The Mycoplasma sensor cells detect the presence of mycoplasmas leading to a color change of the HEK-Blue™ Detection medium. The Mycoplasma sensor cells recognize mycoplasmas through Toll-Like Receptor 2 (TLR2), a pathogen recognition receptor. In the presence of mycoplasmas, TLR2 initiates a signaling cascade leading to the activation of NF-κB and other transcription factors. These transcription factors induce the secretion of SEAP (secreted embryonic alkaline phosphatase) in the supernatant which is readily detected by the purple/blue coloration of the HEK-Blue™ Detection medium.

- **Simple** - Requires only basic cell culture knowledge. Results are easily determined with the naked eye or quantified with a spectrophotometer.
- **Rapid** - Hands-on time less than 1 hour. Gives results after overnight incubation.
- **Versatile** - Detects all *Mycoplasma* and *Acholeplasma* species known to infect cell cultures.
- **Sensitive** - Detects 5.10^2-5.10^5 cfu/ml mycoplasmas. No false positive.
- **Complete** - Contains the Mycoplasma sensor cells and all the reagents needed to perform the assay, including positive and negative controls.



Plasmocin™ & Plasmocure™ - Mycoplasma Elimination Guaranteed!

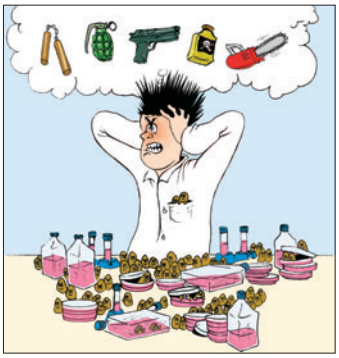
Once the mycoplasma contamination has been confirmed, it is usually recommended to discard the infected cell culture. However, some cell lines are irreplaceable and require an effective eradication treatment. InvivoGen offers two potent mycoplasma removal agents that will guarantee the elimination of mycoplasma without affecting your cell cultures.

Introducing Plasmocure™

Plasmocin™ is a well-established antimycoplasma reagent. Plasmocin™ contains two antibiotics strongly active against mycoplasmas that allow their elimination in only 2 weeks without affecting the cell cultures. Many cell lines infected by mycoplasmas have been successfully treated with Plasmocin™, including embryonic stem cells, hybridomas and retrovirus packaging cells.

In very rare cases, mycoplasmas resistant to Plasmocin™ have been reported. To eradicate these mycoplasmas, InvivoGen has developed a new antimycoplasma agent called Plasmocure™. Plasmocure™ combines two antibiotics that act through different mechanisms of action than those in Plasmocin™. A two week treatment with Plasmocure™ was found sufficient to completely eliminate mycoplasmas. A moderate toxicity can be observed during the course of the treatment but full recovery of the cell line is expected once mycoplasmas are eliminated.

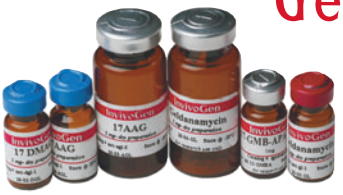
Plasmocin™ and Plasmocure™ are provided as ready-to-use solutions. Simply add to mycoplasma contaminated cell cultures for 2 weeks at the recommended concentration (25 µg/ml for Plasmocin™ and 50 µg/ml for Plasmocure™). Plasmocin™ is provided at a concentration of 25 mg/ml and Plasmocure™ at 100 mg/ml.



Don't let mycoplasma drive you crazy!

Product	Quantity	Catalog Code
PlasmoTest™	1 kit	rep-pt
Plasmocin™	50 mg	ant-mpt
Plasmocure™	100 mg	ant-pc

Geldanamycin & Analogues



Geldanamycin (GA) , a benzoquinone ansamycin antibiotic, interferes with the action of the heat shock protein 90 (Hsp90) leading to the degradation of Hsp90 client proteins. Since many of these client proteins are oncogenic proteins, GA inhibits the proliferation of cancer cells and shows anti-cancer activity in experimental animals. However due to poor aqueous solubility and liver toxicity, GA has not moved forward in clinical trials. To overcome these undesirable properties, numerous GA analogues have been synthesized which differ only in their 17-substituent. These include 17-allylamino-demethoxygeldamycin (17-AAG) and 17-dimethylaminogeldanamycin (17-DMAG) that have completed phase I and are currently entering phase II clinical trials. InvivoGen provides Geldanamycin and five of its more promising analogues as well as other derivatives.

Product	Description	17-Substituent	Solubility	Quantity*	Code
Geldanamycin	Produced by *Streptomyces hygroscopicus*	OCH_3	DMSO (1 mg/ml)	1 mg	ant-gl-1
17-AAG	Less toxic and more stable than GA	$NHCH_2CHCH_2$	DMSO (1 mg/ml)	1 mg	ant-agl-1
17-DMAG§	Water soluble and less toxic than GA	$NHCH_2CH_2N(CH_3)_2$	H_2O (10 mg/ml)	1 mg	ant-dgl-1
17-AEP-GA	Water soluble and less toxic than GA	$NHCH_2CH_2NC_4H_8$	H_2O (10 mg/ml)	1 mg	ant-egl-1
17-DMAP-GA	Water soluble and less toxic than GA	$NHCH_2CH_2CH_2N(CH_3)_2$	H_2O (10 mg/ml)	1 mg	ant-mgl-1
17-GMB-APA-GA	For conjugation to a monoclonal antibody	-	DMSO (1 mg/ml)	1 mg	gmbapa-ga
Biotin-GA	Useful for affinity purification of Hsp90 clients proteins	-	DMSO (1 mg/ml)	1 mg	ant-bl-1
FITC-GA	Useful to identify new Hsp90 inhibitors	-	DMSO (1 mg/ml)	1 mg	ant-fl-1

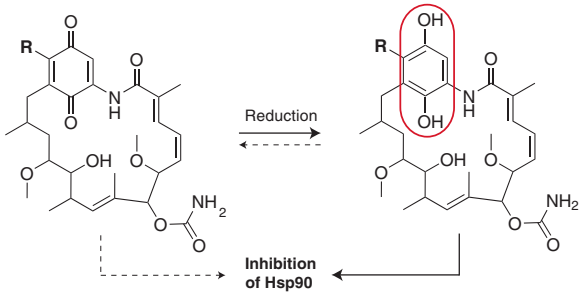
* Also provided in 5 mg - **Bulk quantities readily available**

§ The use of 17-DMAG is covered under US Patent 6,890,917 owned and licensed by the NIH to InvivoGen.

17-AAGH2 and 17-DMAGH2 - Hydroquinone GA Analogues

NEW

The selective toxicity of benzoquinone ansamycins in tumor cells can be explained by their reduction to hydroquinones. Indeed, many human cancer cells express at high levels NAD(P)H:quinone oxidoreductase 1 (NQO1), a flavoenzyme that catalyzes the direct reduction of quinones to hydroquinones. NQO1 has been shown to reduce 17-AAG and to increase the sensitivity to 17-AAG of cancer cell lines[1]. Recent studies have demonstrated that the reduction product of 17-AAG, 17-AAGH2, is a more potent inhibitor of Hsp90 than the 17-AAG itself[2], as well as 17-DMAGH2 compared to 17-DMAG[3].



InvivoGen provides 17-AAGH2 and 17-DMAGH2 produced by reduction of 17-AAG and 17-DMAG respectively using a chemical reducing agent. Both products are supplied as lyophilized light purple powder.

1. Kelland LR. et al., 1999. DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst. 91(22):1940-9.
2. Guo W. et al., 2005. Formation of 17-AAG hydroquinone by NAD(P)H:quinone oxidoreductase 1: role of 17-AAG hydroquinone in heat shock protein 90 inhibition. Cancer Res. 65(21):10006-15.
3. Guo W. et al., 2006. The bioreduction of a series of benzoquinone ansamycins by NAD(P)H:quinone oxidoreductase 1 to more potent heat shock protein 90 inhibitors, the hydroquinone ansamycins. Mol Pharmacol. 70(4):1194-203.

Product	Quantity	Catalog Code
17-AAGH2	1 mg	ant-agh-1
17-DMAGH2	1 mg	ant-dgh-1

InvivoGen™
www.invivogen.com