

SUMMARY :

REVIEW

The RIG-I and STING Alliance

PRODUCTS

RIG-I Pathway

- RIG-I agonists
- RIG-I/MAVS reporter cells

STING Variants

- SAVI STING reporter cells
- Recombinant human STING

Nucleic Acid Complexing Agent

- LyoVec™

Selective Antibiotics

- Cell culture-grade antibiotics

The RIG-I and STING Alliance

The innate immune system is crucial to limit viral infections. It relies on several groups of pattern recognition receptors (PRRs) that recognize viral nucleic acids¹. These PRRs include the cytosolic DNA sensor (CDS), cyclic GMP-AMP synthase (cGAS), and the cytoplasmic RNA sensor, retinoic acid inducible gene I (RIG-I). Once activated, they induce different signaling pathways leading to the production of a variety of antiviral molecules. Interestingly, evidence suggests that these signaling pathways are tightly interconnected to potentiate the antiviral responses.

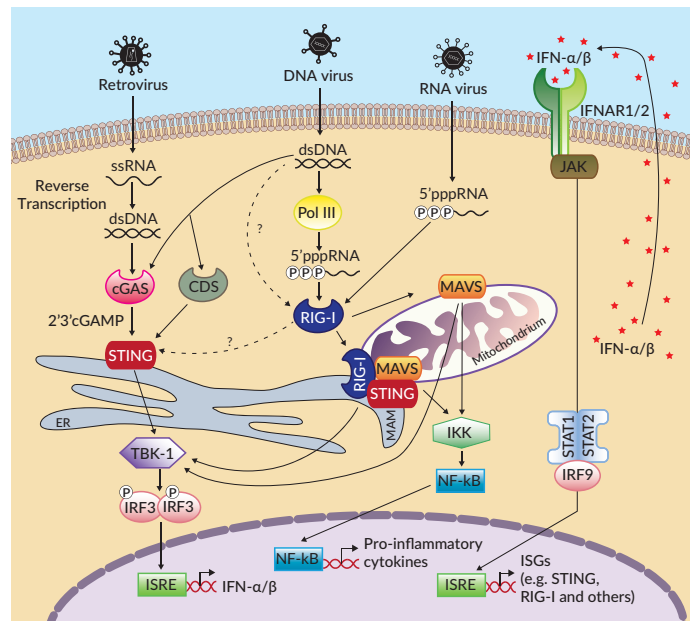
The sensors cGAS and RIG-I recognize different nucleic acids and signal through distinct adaptors. cGAS senses the aberrant presence and concentration of DNA in the cytosol then recruits stimulator of interferon genes (STING, or MITA/ERIS/MPYS) through the cyclic dinucleotide 2'3'cGAMP². RIG-I detects viral RNAs that exhibit an uncapped 5'-di/triphosphate end and a short blunt-ended double stranded (ds) portion, then binds to mitochondrial antiviral signaling protein (MAVS, or IPS-1/VISA/Cardif)¹. Following their activation, cGAS/STING and RIG-I/MAVS trigger common signaling cascades that lead to the production of type I interferons (IFNs) and pro-inflammatory cytokines.

A complex interplay between the RNA and DNA pathways has been reported at different levels. First, several groups have demonstrated that, in human cells but not in mouse cells, cytosolic AT-rich DNA induces type I IFNs in a RIG-I/MAVS-dependent manner³⁻⁵. This recognition by RIG-I was shown to require the transcription of poly(dA:dT) by RNA polymerase III^{4,5}, although a direct interaction between RIG-I and dsDNA has also been suggested⁶. Further, the involvement of STING in the RIG-I/MAVS pathway has been highlighted by the diminished ability of STING-deficient cells to produce type I IFNs in response to cytosolic dsRNA or viral infection and by the direct association of STING and RIG-I in co-immunoprecipitation experiments^{7,8}. These data and others suggest that STING functions as a co-adaptor of activated RIG-I by forming complexes with MAVS at mitochondrial-associated endoplasmic reticulum membranes (MAM)^{7,9}. Additionally, cGAS was shown to participate in the recognition of reverse-transcribed viral RNA¹⁰ and cytosolic RNA:DNA hybrids¹¹, and to bind to synthetic RNA in a crystal structure study¹².

Besides physical interaction between the mediators of the RNA and DNA pathways, coordinated regulation of STING and RIG-I expression levels has been described. Indeed, STING expression is induced upon RIG-I activation by synthetic or viral agonists¹³, and conversely, RIG-I expression can occur downstream of STING activation. In this case, upregulated RIG-I participates in a

negative feedback regulation of STING expression to prevent an excessive immune response¹⁴.

In conclusion, cGAS/STING and RIG-I/MAVS are physically and functionally interconnected. Crosstalk in RNA and DNA detection and subsequent signaling cascades are critical, not only to cope with the diversity of microbial nucleic acids, but to counteract viral escape mechanisms. Moreover, the interplay between RIG-I and STING may help balancing efficient anti-viral response and cellular integrity. Of note, the RIG-I and STING crosstalks vary greatly among species and cell types, probably reflecting the co-evolution of viruses and their cellular targets. Future development of agonists or inhibitors of the nucleic acid sensing pathways will require scrupulous examination of their impact on each nucleic acid sensor.



1. Gebhardt A. et al., 2017. Discrimination of Self and Non-Self Ribonucleic Acids. *Journal of Interferon & Cytokine Research* 37: 184-97. 2. Tao J. et al., 2016. cGAS-cGAMP-STING: The three musketeers of cytosolic DNA sensing and signaling. *IUBMB Life* 68: 858-70. 3. Cheng G. et al., 2007. Double-stranded DNA and double-stranded RNA induce a common antiviral signaling pathway in human cells. *Proceedings of the National Academy of Sciences* 104: 9035-40. 4. Ablasser A. et al., 2009. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol* 10: 1065-72. 5. Chiu Y.-H. et al., 2009. RNA Polymerase III Detects Cytosolic DNA and Induces Type I Interferons through the RIG-I Pathway. *Cell* 138: 576-91. 6. Choi M.K. et al., 2009. A selective contribution of the RIG-I-like receptor pathway to type I interferon responses activated by cytosolic DNA. *Proceedings of the National Academy of Sciences* 106: 17870-5. 7. Zhong B. et al., 2008. The Adaptor Protein MITA Links Virus-Sensing Receptors to IRF3 Transcription Factor Activation. *Immunity* 29: 538-50. 8. Ishikawa H. & Barber GN. 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature*. 455(7213):674-8. 9. Castanier C. et al., 2010. Mitochondrial dynamics regulate the RIG-I-like receptor antiviral pathway. *EMBO reports* 11: 133-8. 10. Gao D. et al., 2013. Cyclic GMP-AMP Synthase Is an Innate Immune Sensor of HIV and Other Retroviruses. *Science* 341: 903-6. 11. Mankan AK. et al., 2014. Cytosolic RNA:DNA hybrids activate the cGAS-STING axis. *EMBO J.* 33(24):2937-46. 12. Civril F. et al., 2013. Structural mechanism of cytosolic DNA sensing by cGAS. *Nature* 498: 332-7. 13. Liu Y. et al., 2016. RIG-I-Mediated STING Upregulation Restricts Herpes Simplex Virus 1 Infection. *Journal of Virology* 90: 9406-19. 14. Wu X. et al., 2017. RIG-I and IL-6 are negative-feedback regulators of STING induced by double-stranded DNA. *PLOS ONE* 12: e0182961.



RIG-I Pathway

RIG-I Ligands

• 5'ppp-dsRNA

5'ppp-dsRNA is a 5' triphosphate double-stranded RNA obtained by hybridization of one 5' triphosphate single-stranded 19-mer phosphodiester RNA with its non-triphosphate complementary strand. The sequence for 5'ppp-dsRNA was determined following screening of various sequence variations. This uncapped 5'triphosphate dsRNA is specifically recognized by RIG-I.

• 3p-hpRNA

3p-hpRNA is a 5' triphosphate hairpin RNA that was generated by *in vitro* transcription of a sequence from influenza A (H1N1) virus. This 87-mer RNA oligonucleotide contains an uncapped 5'triphosphate extremity and a double-strand fragment which are the structural features recognized by RIG-I. 3p-hpRNA is a potent and specific agonist of RIG-I.

 www.invivogen.com/rlr-ligands

RIG-I/MAVS Reporter Cells

InvivoGen provides a collection of cell lines derived from the human lung A549 carcinoma, the mouse RAW macrophage and the human embryonic kidney HEK293 cells designed to facilitate the study of the RNA sensing RIG-I pathway. These cells are either knockout for the RIG-I or MAVS gene (A549-derived or RAW-derived cells), or they overexpress the RIG-I gene (HEK293-derived cells).

- A549-Dual™
- RAW-Lucia™ ISG
- HEK-Lucia™ Null
- A549-Dual™ KO-RIG-I
- RAW-Lucia™ ISG KO-RIG-I
- HEK-Lucia™ RIG-I
- A549-Dual™ KO-MAVS
- RAW-Lucia™ ISG KO-MAVS

Description

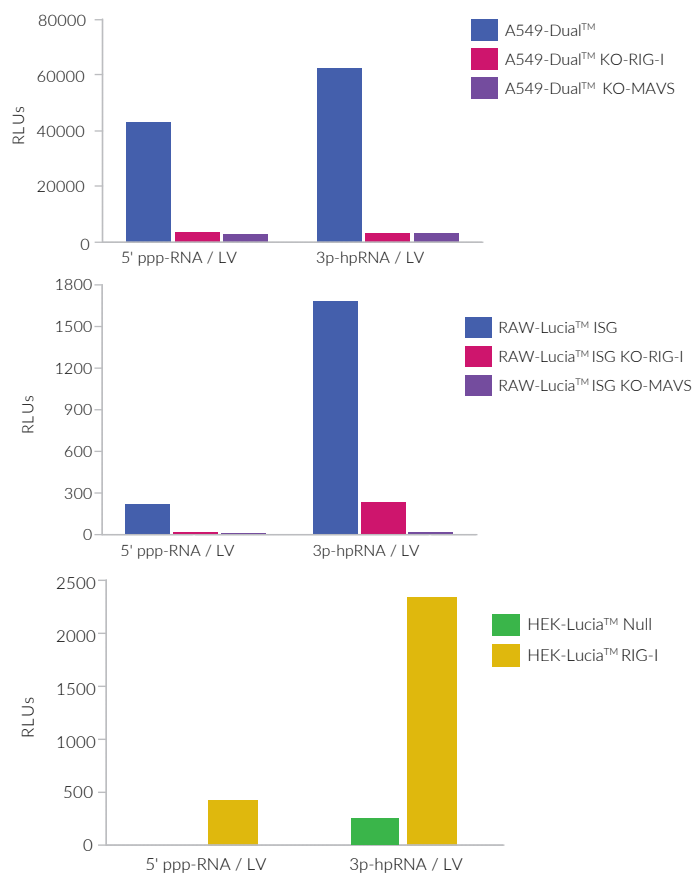
All the RIG-I/MAVS reporter cells express an ISG (interferon stimulated gene)-inducible construct containing the secreted Lucia luciferase gene. Activation of the RIG-I/MAVS pathway induces the activation of the ISG promoter and the production of Lucia luciferase which can be measured in the cell supernatant using the luciferase detection reagent, QUANTI-Luc™. The A549-derived cells carry an additional NF-κB-inducible construct expressing the secreted embryonic alkaline phosphatase (SEAP) gene which activity can be determined using the SEAP detection reagent, QUANTI-Blue™.

The RIG-I/MAVS reporter cells are resistant to Zeocin™ alone or Zeocin™ and blasticidin (see last page).

Results

Stimulation of A549-Dual™ and RAW-Lucia™ ISG cells with the RIG-I ligands, 5'ppp-dsRNA and 3p-hpRNA, complexed to LyoVec™ leads to a significant ISG response. This response is much higher when using 3p-hpRNA compared to 5'ppp-dsRNA, especially in A549-Dual™ cells in which an equal activity is observed with 30 ng/ml of complexed 3p-hpRNA or 300 ng/ml of complexed 5'ppp-dsRNA. In contrast, in the RIG-I-KO or MAVS-KO cell lines this response is strongly diminished. Stimulation of HEK-Lucia™ Null cells with RIG-I ligands results in a weak ISG response, whereas in HEK-Lucia™ RIG-I cells, this response is much stronger due to the constitutive overexpression of the RIG-I gene. Again, a higher ISG response is observed when using 3p-hpRNA versus 5'ppp-dsRNA. The NF-κB response to RIG-I ligands in A549-derived cells is similar to the ISG response although weaker (see data on website).

 www.invivogen.com/rlr-cell-lines



ISG Responses to RIG-I ligands: A549-derived cells were stimulated with 300 ng/ml 5'ppp-dsRNA or 30 ng/ml 3p-hpRNA, while RAW- and HEK-derived cells were induced with 1 µg/ml 5'ppp-dsRNA or 3p-hpRNA. After overnight incubation, the ISG response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ expressed as relative light units (RLUs).

PRODUCT	QUANTITY	CAT. CODE
A549-Dual™ cells	3-7 x 10 ⁶ cells	a549d-nfis
A549-Dual™ KO-MAVS cells	3-7 x 10 ⁶ cells	a549d-komavs
A549-Dual™ KO-RIG-I cells	3-7 x 10 ⁶ cells	a549d-korigi
RAW-Lucia™ ISG cells	3-7 x 10 ⁶ cells	rawl-isg
RAW-Lucia™ ISG-KO-MAVS cells	3-7 x 10 ⁶ cells	rawl-komavs
RAW-Lucia™ ISG-KO-RIG-I cells	3-7 x 10 ⁶ cells	rawl-korigi
HEK-Lucia™ Null cells	NEW 3-7 x 10 ⁶ cells	hkl-null
HEK-Lucia™ RIG-I cells	NEW 3-7 x 10 ⁶ cells	hkl-hrigi
5'ppp-dsRNA	25 µg	ttrl-3prna
3p-hpRNA	NEW 25 µg	ttrl-hprna

RELATED PRODUCTS

PRODUCT	DESCRIPTION	CAT. CODE
QUANTI-Luc™	Luciferase detection reagent	rep-qlc1
QUANTI-Blue™	SEAP detection reagent	rep-qb1

For more information, please visit : www.invivogen.com

STING Variants

STING SAVI Reporter Cells

InvivoGen provides a collection of STING variant reporter cells derived from the human THP-1 monocytic cell line. Among them, two express a STING gain-of-function (S154 and M155) responsible for the STING-associated vasculopathy with onset in infancy (SAVI). SAVI-patients display a single-point mutation in the STING protein leading to its constitutive activation and excessive activation of ISGs. While S154 results from a *de novo* germline mutation¹, M155 is an inherited mutation². STING SAVI reporter cells are convenient and powerful tools for antagonist screening in the STING pathway.

- THP1-Dual™ KI-hSTING-S154
- THP1-Dual™ KI-hSTING-M155

Description

THP1-Dual™ KI-hSTING-S154 cells and THP1-Dual™ KI-hSTING-M155 cells were generated from THP1-Dual™ KO-STING cells, which derive from the THP-1 cell line by stable biallelic knockout of the endogenous human STING gene, and stable integration of two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). THP1-Dual™ KO-STING cells were knocked in with the intronless coding sequence of the “wild-type” R232 hSTING variant³ and a S154 or M155 point mutation. The IRF3 and NF-κB pathways can be examined by monitoring the activity of Lucia luciferase and SEAP respectively. Activity of the reporter proteins can be monitored in the cell culture supernatant using QUANTI-Luc™ and QUANTI-Blue™ detection reagents. The SAVI reporter cells are resistant to blasticidin and Zeocin™.

Results

Gain-of-function activation - Both THP1-Dual™ KI-hSTING-S154 cells and THP1-Dual™ KI-hSTING-M155 cells display a constitutive ISG response while THP-1-Dual™ KI-hSTING-R232 cells do not. Addition of IFN-β or STING agonists further exacerbates the ISG response. Of note, the results are normalized on the background signal in non-induced (NI) cells. The background of SAVI cell lines being extremely high due to constitutive STING activation, the fold-increase in the ISG response upon induction is lower than with the R232 control cells.

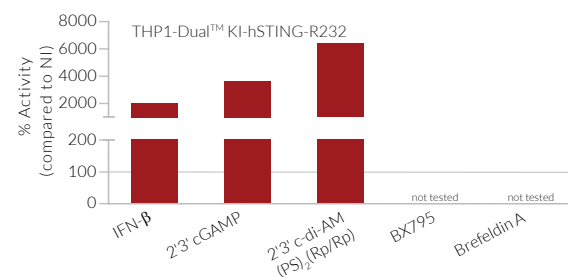
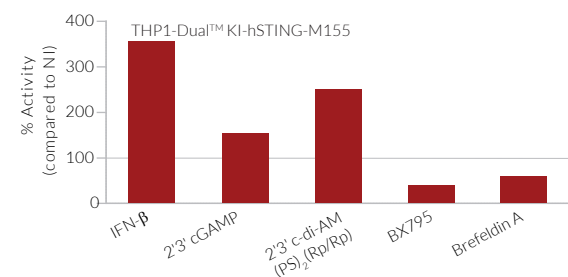
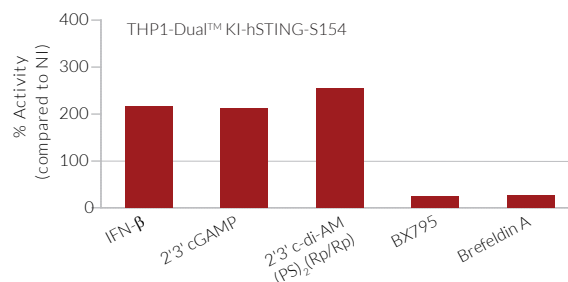
Responses to inhibitors - Addition of BX795, a TBK-1 inhibitor, or Brefeldin A, an inhibitor of protein transport from the ER to the Golgi apparatus, significantly impedes STING signaling in the SAVI cell lines. Thus, these cells can be used to screen for STING antagonists.

1. Munoz J. et al., 2015. Stimulator of interferon genes-associated vasculopathy with onset in infancy: a mimic of childhood granulomatosis with polyangiitis. *JAMA Dermatology* 151: 872-7.
2. Jeremiah N. et al., 2014. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. *The Journal of Clinical Investigation* 124: 5516-20.
3. Yi G. et al., 2013. Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. *PLOS ONE* 8: e77846.

PRODUCT	QUANTITY	CAT. CODE
THP1-Dual™ KI-hSTING-S154 (SAVI) cells	NEW 3-7 x 10 ⁶ cells	thpd-s154
THP1-Dual™ KI-hSTING-M155 (SAVI) cells	NEW 3-7 x 10 ⁶ cells	thpd-m155

RELATED PRODUCTS

PRODUCT	DESCRIPTION	CAT. CODE
THP1-Dual™ KI-hSTING-R232 cells	R232 variant- (“wild-type”) expressing cells	thpd-r232
2'3'-cGAMP	Cyclic dinucleotide	tlrl-nacga23
2'3'-c-di-AM(PS) ₂ (Rp,Rp)	Cyclic dinucleotide	tlrl-nacda2r-01



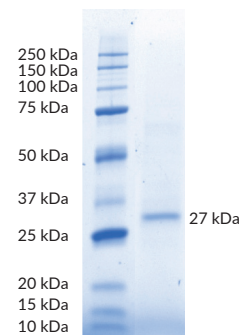
ISG Responses to IFN-β or STING ligands: THP1-Dual™-derived cells were stimulated with 10⁴ U/ml IFN-β, 10 μg/ml 2'3' cGAMP or 2'3' cGAMP(PS)₂(Rp/Sp), 30 μM BX795 or 10 μg/ml Brefeldin A. After overnight incubation, the ISG response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Bars represent the % activity normalized on the background signal in non-induced (NI) cells.

STING Screening Services

InvivoGen offers an extensive choice of STING screening services for the identification of molecules that induce or inhibit a number of STING variants. **Contact us for more information.**

Recombinant Human STING

STING binds to CDNs through its cytoplasmic domain. InvivoGen provides a recombinant human STING protein that corresponds to the soluble cytoplasmic domain (aa 137-379) of the R232 variant, which is the most prevalent human STING isoform. This recombinant protein of 27 kDa is produced in the mammalian Chinese hamster ovary (CHO) cell line and purified by affinity chromatography. It contains no tag. It is recognized by commercially available antibodies to human STING.



PRODUCT	QUANTITY	CAT. CODE
Recombinant Human STING NEW	25 μg	rec-hsting

Nucleic Acid Complexing Agent

LyoVec™

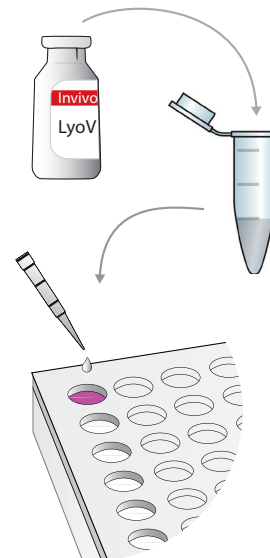
LyoVec™ is the first lyophilized cationic lipid-based transfection reagent. It consists of the phosphonolipid DTCPTA, which is coupled with DiPPE, a neutral lipid that helps destabilizing membrane bilayers, therefore increasing the *in vitro* transfection efficiency of LyoVec™. The positive charge of LyoVec™ enables it to bind to DNA, and its phospholipid structure promotes fusion with cellular membranes for DNA delivery.

LyoVec™, which was developed as a plasmid DNA transfection reagent, can be effectively used as a nucleic acid complexing agent to facilitate the entry within the cells of RNA or DNA-based oligonucleotides, such as the RIG-I ligands, 5'ppp-dsRNA and 3p-hpRNA (see second page) or the cGAS ligands, HSV60 and VACV70. The complexation step is crucial to obtain a response to nucleic acids induced by pathogen recognition receptors.

PRODUCT	QUANTITY	CAT. CODE
LyoVec™	10 ml (200 reactions)	lyec-1
LyoVec™	20 ml (400 reactions)	lyec-2

 www.invivogen.com/transfection-reagents

Procedure



01

Reconstitute lyophilized LyoVec™ with 2 ml of sterile H₂O or PBS

02

Prepare complex at 10 µg/ml:
 - In a 1.5 ml tube, add first 1 µl ligand @ 1 mg/ml
 - Add drop by drop 100 µl of LyoVec™
 - Homogenize gently by tapping the tube
 - Incubate for 15-30 minutes at room temperature

Prepare a dilution range

03

Dispense 20 µl of complex in a well of a 96-well plate containing 180 µl of cell suspension

Selective Antibiotics

Cell-culture tested antibiotics

- Sterile
- Endotoxin-free
- Functionally validated

InvivoGen offers a range of cell-culture tested antibiotics to ensure artifact-free selection of transfected mammalian cells. These antibiotics are sterile and endotoxin-free to avoid the deleterious effects of bacterial endotoxins, also known as lipopolysaccharides (LPS), on transfected cells. They are functionally validated through rigorous physico-chemical, microbiological and cellular testing. They exhibit proven long-term stability to mammalian cells with no cytotoxicity.

InvivoGen's selective antibiotics can be used in combination with other selective antibiotics or with antimicrobial antibiotics designed to prevent mycoplasmal, bacterial or fungal contaminations of cell cultures. They are available in different sizes.

RELATED PRODUCTS

PRODUCT	DESCRIPTION	CAT. CODE
Fungin™	Anti-fungal agent	ant-fn-1
Normocin™	Anti-microbial agent	ant-nr-1
Plasmocin™	Anti-mycoplasma agent	ant-mpp

Selective antibiotic	Resistance gene	Concentration in bacteria	Concentration in mammalian cells
Blasticidin	<i>bsr</i> gene	25-100 µg/ml	1-10 µg/ml
G418 (Geneticin)	<i>neo</i> gene	-	400-1000 µg/ml
Hygromycin B Gold	<i>hph</i> gene	50-100 µg/ml	50-200 µg/ml
Puromycin	<i>pac</i> gene	-	1-10 µg/ml
Zeocin™	<i>Sh ble</i> gene	25 µg/ml	50-400 µg/ml

PRODUCT	QUANTITY	CAT. CODE
Blasticidin	100 mg (10 x 1 ml)	ant-bl-1
G418 (Geneticin)	1 g (10 x 1 ml)	ant-gn-1
Hygromycin B Gold	1 g (10 x 1 ml)	ant-hg-1
Puromycin	100 mg (10 x 1 ml)	ant-pr-1
Zeocin™	1 g (10 x 1 ml)	ant-zn-1

 www.invivogen.com/selective-antibiotics

