

# Hygromycin B Gold

Selection antibiotic; cell culture tested  
Catalog code: ant-hg-1, ant-hg-2, ant-hg-5  
<http://www.invivogen.com/hygromycin>

For research use only

Version 19K29-MM

## PRODUCT INFORMATION

### Contents:

Hygromycin B Gold (previously named HygroGold™) is an ultrapure Hygromycin B. It is supplied as a sterile filtered yellow solution at 100 mg/ml solution in HEPES buffer. It is available in 3 pack sizes:

- **ant-hg-1:** 10 x 1 ml (1 g)
- **ant-hg-2:** 20 x 1 ml (2 g)
- **ant-hg-5:** 1 x 50 ml (5 g)

### Storage and stability:

- Hygromycin B Gold is shipped at room temperature. Upon receipt, it should be stored at 4°C or -20°C. Avoid repeated freeze-thaw cycles.
- The expiry date is specified on the product label.
- Hygromycin B Gold is sensitive to high concentrations of acid but can tolerate brief exposure to dilute acids.
- Protect Hygromycin B Gold from light.

**Note:** Hygromycin B Gold is stable for 3 months at room temperature.

## QUALITY CONTROL

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Purity: ≥ 90% (HPLC)
- Endotoxin level: < 0.5 EU/mg
- Physicochemical characterization (pH, appearance)
- Cell culture tested: potency validated in hygromycin-sensitive and hygromycin-resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in hygromycin-resistant cells

## DESCRIPTION

Hygromycin B is a selection antibiotic that acts on both eukaryotic and prokaryotic cells. It is an aminoglycoside antibiotic produced by *Streptomyces hygrosopicus*. It kills eukaryotic and prokaryotic cells through the inhibition protein synthesis. More specifically, it has been reported to interfere with translocation<sup>1</sup> and to cause mistranslation at the 70S ribosome<sup>2</sup>.

Resistance to hygromycin is conferred by the *hph* gene.

## SAFETY CONSIDERATIONS

Hygromycin B Gold is a harmful compound. Refer to safety data sheet for handling instructions.

## GENERAL GUIDELINES

Successful transfection is influenced by many factors. The health and viability of the cell line, the quality of the nucleic acid used, the transfection reagent, the duration of transfection, and the presence or absence of serum can all play a part.

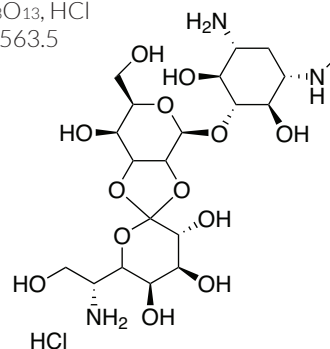
## CHEMICAL PROPERTIES

CAS number: 31282-04-9

Formula: C<sub>20</sub>H<sub>37</sub>N<sub>3</sub>O<sub>13</sub>, HCl

Molecular weight: 563.5

Structure:



## SELECTION CONDITIONS

Most cells growing aerobically are killed by Hygromycin B Gold in the concentration range of 50 to 500 µg/ml. However, the sensitivity of cells is pH dependent (i.e. the higher the pH of the culture medium the greater the sensitivity). Thus, the concentration of Hygromycin B Gold required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, you can also lower the required amount of Hygromycin B Gold by using low-salt media whenever possible.

### - *Escherichia coli*

Hygromycin-resistant transformants are selected in low-salt LB agar medium (yeast extract 5g/l, tryptone 10 g/l, NaCl 5 g/l, agar 15 g/l, pH 8) supplemented with 50 to 100 µg/ml of Hygromycin B Gold. Plates containing Hygromycin B Gold are stable for 1 month when stored at 4 °C.

### - Mammalian cells

The working concentrations of Hygromycin B Gold for mammalian cell lines vary from 50 to 200 µg/ml; in a few cases, up to 500 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Hygromycin B Gold required to kill your host cell line. Killing and detachment of dead cells from the plate, especially at high cell density, can require a longer time than with G418. Hygromycin-resistant stable transfectants are usually obtained after 10 days to 3 weeks incubation, depending on the cell line. See table on the next page for suggested working concentrations of Hygromycin B Gold in mammalian cells.

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

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## WORKING CONCENTRATIONS

Hygromycin B Gold is normally used at a concentration of 200 µg/ml, a 500-fold dilution from the stock solution. However, the optimal concentration needs to be determined for your cells. Suggested concentrations of Hygromycin B Gold for selection in some examples of mammalian cells are listed below.

Cell line	Medium	Hygromycin B conc.	Ref.
B16 (Mouse melanocytes)	RPMI	100-200 µg/ml	3, 4
CHO (Chinese hamster ovarian cells)	MEM	100-500 µg/ml	5-7
HeLa (Human uterine cells)	DMEM	100-200 µg/ml	8, 9
HEK293 (Human embryonic kidney cells)	DMEM	50-400 µg/ml	10-12
Raji (Human lymphocytes)	RPMI	125-300 µg/ml	13, 14
THP-1 (Human monocytes)	RPMI	250-400 µg/ml	15, 16

## REFERENCES

1. Cabanas M. *et al.*, 1978. Dual interference of Hygromycin B with ribosomal translocation and with Aminoacyl-tRNA recognition. *Eur. J. Biochem.* 87:21-7. 2. Gonzales, A. *et al.*, 1978. Studies on the mode of action of hygromycin B, an inhibitor of translocation in eukaryotes. *Biochem Biophys Acta* 521:459-69. 3. Neerinx A. *et al.*, 2012. NLRC5 controls basal MHC class I gene expression in an MHC enhanceosome-dependent manner. *J Immunol.* 188(10):4940-50. 4. Saccheri F. *et al.*, 2010. Bacteria-induced gap junctions in tumors favor antigen cross-presentation and antitumor immunity. *Sci Transl Med.* 2(44):44ra57. 5. Hidmark A. *et al.*, 2012. Cutting edge: TLR13 is a receptor for bacterial RNA. *J Immunol.* 189(6):2717-21. 6. Hennen S. *et al.*, 2013. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. *Sci Signal.* 6(298):ra93. 7. Raschi E. *et al.*, 2014. β-2-glycoprotein I, lipopolysaccharide and endothelial TLR4: Three players in the two hit theory for anti-phospholipid-mediated thrombosis. *J Autoimmun.* S0896-8411(14)00065-1. 8. Ravid D. *et al.*, 2010. A role for caveolin 1 in assembly and budding of the paramyxovirus parainfluenza virus 5. *J Virol.* 4(19):9749-59. 9. Hayashi Y. *et al.*, 2014. Mechanism by which a LINE protein recognizes its 3' tail RNA. *Nucleic Acids Res.* 42(16):10605-17. 10. Bowen WS. *et al.*, 2012. Selective TRIF-dependent signaling by a synthetic Toll-like receptor 4 agonist. *Sci Signal.* 5(211):ra13. 11. Leskela TT. *et al.*, 2012. Cys-27 variant of human [delta]-opioid receptor modulates maturation and cell surface delivery of Phe-27 variant via heteromerization. *J. Biol. Chem.*, 287: 5008-20. 12. van den Bosch MW. *et al.*, 2014. LPS induces the degradation of programmed cell death protein 4 (PDCD4) to release Twist2, activating c-Maf transcription to promote interleukin-10 production. *J Biol Chem.* 289(33):22980-90. 13. Benjamin LR. *et al.*, 2008. Hierarchical mechanisms build the DNA-binding specificity of FUSE binding protein. *PNAS.* 105(47):18296-301. 14. Bornkamm GW. *et al.*, 2005. Stringent doxycycline-dependent control of gene activities using an episomal one-vector system. *Nucleic Acids Res.* 33(16):e137. 15. Bouwman LI. *et al.*, 2014. Inflammasome Activation by *Campylobacter jejuni*. *J Immunol.* 193(9):4548-57. 16. Bryan NB. *et al.*, 2009. Activation of inflammasomes requires intracellular redistribution of the apoptotic speck-like protein containing a caspase recruitment domain. *J Immunol.* 182(5):3173-82.

## RELATED PRODUCTS

Product	Description	Catalog Code
<b>Other selection antibiotics</b>		
Blasticidin	Selection antibiotic for the <i>bsr</i> or <i>BSD</i> genes	ant-bl-1
G418	Selection antibiotic for the <i>neo</i> gene	ant-gn-1
Puromycin	Selection antibiotic for the <i>pac</i> gene	ant-pr-1
Zeocin™	Selection antibiotic for the <i>Sh ble</i> gene	ant-zn-1
<b>Plasmids encoding the <i>hph</i> gene</b>		
pMOD2-Hygro	Plasmid encoding a synthetic <i>hph</i> gene	pmod2-hygro
pSELECT-hygro-LacZ	LacZ-expression plasmid selectable with hygromycin	pseth-lacZ
pSELECT-hygro-mcs	Expression plasmid selectable with hygromycin	pseth-mcs

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