**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 2 pack sizes:
- ant-hg-1: 10 x 1 ml (1 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 hygroscopicus. It kills eukaryotic and prokaryotic cells through the inhibition protein synthesis. More specifically, it has been reported to interfere with translocation\(^1\) and to cause mistranslation at the 70S ribosome\(^2\). 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.

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**CHEMICAL PROPERTIES**

**CAS number:** 31282-04-9  
**Formula:** C\(_{20}\)H\(_{37}\)N\(_3\)O\(_{13}\), HCl  
**Molecular weight:** 563.5  
**Structure:**

![Chemical Structure](image)

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**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.
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.

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


RELATED PRODUCTS

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<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
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<tbody>
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<td>Blasticidin</td>
<td>Selection antibiotic for the bsr or BSD genes</td>
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<td>G418</td>
<td>Selection antibiotic for the neo gene</td>
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<tr>
<td>Puromycin</td>
<td>Selection antibiotic for the pac gene</td>
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<td>Zeocin®</td>
<td>Selection antibiotic for the Sh ble gene</td>
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<td>Plasmid encoding a synthetic hph gene</td>
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<td>LacZ-expression plasmid selectable with hygromycin</td>
<td>pseth-lacz</td>
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<tr>
<td>pSELECT-hygro-mcs</td>
<td>Expression plasmid selectable with hygromycin</td>
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TECHNICAL SUPPORT

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