Zeocin® Powder
Selective antibiotic for the Sh ble gene; cell culture tested
Catalog code: ant-zn-1p, ant-zn-5p
http://www.invivogen.com/zeocin

For research use only
Version 22G21-MM

PRODUCT INFORMATION
Contents
Zeocin® is supplied as a blue powder.
• ant-zn-1p: 1 x 1 g tube
• ant-zn-5p: 1 x 5 g bottle
Note: Zeocin® powder is very hygroscopic. Keep tubes tightly closed after each use.

Storage and stability
- Zeocin® is shipped at room temperature. Upon receipt it should be stored at 4°C. The expiry date is specified on the product label.
- Store Zeocin® solutions at 4°C or -20°C. Do not use Zeocin® solutions after the expiry date on the product label. Avoid repeated freeze-thaw cycles.
- Zeocin® is sensitive to high concentrations of acids and bases but a short-term exposure to dilute acids can be tolerated.

QUALITY CONTROL
Each lot is tested to ensure the absence of lot-to-lot variation.
- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (including HPLC, pH, appearance)
- Cell culture tested: potency validated in Zeocin®-sensitive and Zeocin®-resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in Zeocin®-resistant cells

BACKGROUND
Zeocin® is a selective antibiotic that acts on both eukaryotic and prokaryotic cells. Resistance to Zeocin® is conferred by the Sh ble gene from Streptomyces hindustanii 1-3. Zeocin® is the commercial name for a special formulation containing Phleomycin, a copper-chelated glycopeptide antibiotic isolated from a mutant strain of Streptomyces verticillus. This antibiotic of the bleomycin family exhibits activity against bacteria, eukaryotic microorganisms, plant and animal cells. Although bleomycin antibiotics perturb plasma membranes, their activity is generally believed to be related to their ability to bind and intercalate DNA thus destroying the integrity of the double helix.

CHEMICAL PROPERTIES
Zeocin® is a mixture of structurally related antibiotics which differ by their terminal amine residues. The antibiotics are in a copper chelated form giving the solution a blue color. Zeocin® is a labile compound which undergoes irreversible denaturation at high and low pH or in presence of a weak oxidant. Zeocin® is freely soluble in water (>500 mg/ml) forming a blue solution.

SAFETY CONSIDERATIONS
Zeocin® is a harmful compound. Refer to safety data sheet for handling instructions.

METHOD
Preparation of Zeocin solution
1. Resuspend Zeocin® in HEPES buffer (5 g/L, pH 7.2+/- 0.1) at a concentration of 100 mg/ml.
2. Sterile filter the solution using a 0.22 µm sterile filter.
3. Store at 4°C for 12 months or -20°C for 18 months.

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.

CONDITIONS OF SELECTION
Most cells growing aerobically are killed by Zeocin® in the concentration range of 0.5 to 1000 µg/ml. However, the sensitivity of cells is pH dependent, i.e. the higher the pH of culture medium, the greater the sensitivity. Thus, the concentration of Zeocin® required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of Zeocin® is reduced by a factor of two to three in hypertonic media, such as those used for protoplast regeneration. Thus, using low salt media when possible decreases the amount of Zeocin® needed.

- *Escherichia coli*
  The Sh ble gene and the hybrid genes in vectors provided by InvivoGen are driven by synthetic E. coli promoters (i.e. EM7). The cells of the common E. coli recipient strains (i.e. HB101, DH5α, MC1061) transformed by these vectors are resistant to Zeocin®.
  
  Note: Do not use an E. coli recipient strain that contains the Tn5 transposable element (i.e. MC1066). Tn5 encodes a bleomycin-resistance gene that will confer resistance to Zeocin®.

Zeocin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5 g/L, Tryptone 10 g/L, NaCl 5 g/L, Agar 15 g/L, pH 7.5) supplemented with 25-50 µg/ml of Zeocin®. Plates containing Zeocin® are stable for 1 month when stored at 4°C.

- *Mammalian cells*
  The working concentration of Zeocin® for mammalian cell lines varies from 50 to 400 µg/ml, in a few cases can be as low as 20 µg/ml or as high as 1000 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Zeocin® required to kill your host cell line. The killing and the detachment of dead cells from the plate, especially at high cell density, can require a longer time compared to G418. Foci of Zeocin-resistant stable transfectants are usually individualized after 5 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of Zeocin® for selection in mammalian cells are listed on the next page.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

Any questions about our selective antibiotics? Visit our FAQ page.
**Working Concentrations**

Zeocin® is normally used at a concentration of 100 µg/ml, a 1000-fold dilution from the stock solution. However, the optimal concentration needs to be determined for your cells. Suggested concentrations of Zeocin® for selection in some examples of mammalian cells are listed below.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Medium</th>
<th>Zeocin® conc (µg/ml)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 (Mouse melanocytes)</td>
<td>RPMI</td>
<td>20-250</td>
<td>4-6</td>
</tr>
<tr>
<td>CHO (Chinese hamster ovarian cells)</td>
<td>DMEM</td>
<td>100-500</td>
<td>4, 7, 8</td>
</tr>
<tr>
<td>COS (Monkey kidney cells)</td>
<td>DMEM</td>
<td>100-400</td>
<td>9, 10</td>
</tr>
<tr>
<td>HEK293 (Human embryonic kidney cells)</td>
<td>DMEM</td>
<td>100-400</td>
<td>11, 12</td>
</tr>
<tr>
<td>HeLa (Human uterine cells)</td>
<td>DMEM</td>
<td>50-100</td>
<td>13, 14</td>
</tr>
<tr>
<td>J558L (Mouse melanocytes)</td>
<td>RPMI</td>
<td>400</td>
<td>15</td>
</tr>
<tr>
<td>MCF-7 (Mouse breast adenocarcinoma cells)</td>
<td>DMEM</td>
<td>100-400</td>
<td>16, 17</td>
</tr>
<tr>
<td>MEFs (Mouse embryonic fibroblasts)</td>
<td>DMEM</td>
<td>200-400</td>
<td>18, 19</td>
</tr>
<tr>
<td>THP-1 (Human monocytes)</td>
<td>RPMI</td>
<td>200 µg/ml</td>
<td>20</td>
</tr>
</tbody>
</table>

**References**


**Related Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other selective antibiotics</td>
<td>Selective antibiotic for the bsr or BSD genes</td>
<td>ant-bl-1</td>
</tr>
<tr>
<td>Blasticidin</td>
<td>Selective antibiotic for the neo gene</td>
<td>ant-gn-1</td>
</tr>
<tr>
<td>G418</td>
<td>Selective antibiotic for the hph gene</td>
<td>ant-hg-1</td>
</tr>
<tr>
<td>Hygromycin B Gold</td>
<td>Selective antibiotic for the pac gene</td>
<td>ant-pr-1</td>
</tr>
<tr>
<td>Puromycin</td>
<td>Plasmid encoding a synthetic Sh ble gene</td>
<td>pmod2-zeo</td>
</tr>
<tr>
<td>Plasmids encoding the Sh ble gene</td>
<td>LacZ-expression plasmid selectable with Zeocin®</td>
<td>psetz-lacz</td>
</tr>
<tr>
<td>pMOD2-Zeo</td>
<td>Expression plasmid selectable with Zeocin®</td>
<td>psetz-mcs</td>
</tr>
</tbody>
</table>