**Zeocin™**

Selection antibiotic for the Sh ble gene; cell culture tested

Catalog # ant-zn-1, ant-zn-5, ant-zn-5b

http://www.invivogen.com/zeocin

For research use only

Version # 16D20-MM

---

**PRODUCT INFORMATION**

**Contents**

Zeocin™ is supplied a sterile filtered blue solution at 100 mg/ml in HEPES buffer.

- **ant-zn-1:** 10 x 1 ml (1 g)
- **ant-zn-5:** 50 x 1 ml (5 g)
- **ant-zn-5b:** 1 x 50 ml (5 g)

**Storage and stability**

- Zeocin™ is shipped at room temperature. Upon receipt it should be stored at 4 °C or at -20 °C. Avoid repeated freeze-thaw cycles.
- The expiry date is specified on the product label.
- 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 thoroughly 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 selection antibiotic that acts on both eukaryotic and prokaryotic cells. Resistance to Zeocin™ is conferred by the Sh ble gene from Streptomyces hindustanus. Zeocin™ is the commercial name for a special formulation containing Phleomycin, a copper-chelated glycopeptide antibiotic isolated from a mutant strain of Streptomycetes 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.

**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.

**SAFETY CONSIDERATIONS**

Zeocin™ is a harmful compound. Refer to safety data sheet for handling instructions.

**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.

**CONDITIONS OF SELECTION**

Most cells growing aerobically are killed by 0.5 to 1000 µg/ml Zeocin™. 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 2 to 3 in hypertonics media, such as those used for protoplast regeneration. Hence, using low salt medium 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, DH5a, 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 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, may 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 Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
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</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 (Mouse melanocytes)</td>
<td>RPMI</td>
<td>20-250 µg/ml</td>
<td>4-6</td>
</tr>
<tr>
<td>CHO (Chinese hamster ovarian cells)</td>
<td>DMEM</td>
<td>100-500 µg/ml</td>
<td>4, 7, 8</td>
</tr>
<tr>
<td>COS (Monkey kidney cells)</td>
<td>DMEM</td>
<td>100-400 µg/ml</td>
<td>9, 10</td>
</tr>
<tr>
<td>HEK293 (Human embryonic kidney cells)</td>
<td>DMEM</td>
<td>100-400 µg/ml</td>
<td>11, 12</td>
</tr>
<tr>
<td>HeLa (Human uterine cells)</td>
<td>DMEM</td>
<td>50-100 µg/ml</td>
<td>13, 14</td>
</tr>
<tr>
<td>J558L (Mouse melanocytes)</td>
<td>RPMI</td>
<td>400 µg/ml</td>
<td>15</td>
</tr>
<tr>
<td>MCF-7 (Human breast adenocarcinoma cells)</td>
<td>DMEM</td>
<td>100-400 µg/ml</td>
<td>16, 17</td>
</tr>
<tr>
<td>MEFs (Mouse embryonic fibroblasts)</td>
<td>DMEM</td>
<td>200-400 µg/ml</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

5. Hirose Y. et al., 2012. Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. PNAS. 109: 4263-4268.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other selection antibiotics</strong></td>
<td>Selection antibiotic for the bsr or BSD genes</td>
<td>ant-bl-1</td>
</tr>
<tr>
<td>G418</td>
<td>Selection antibiotic for the neo gene</td>
<td>ant-gn-1</td>
</tr>
<tr>
<td>Hygromycin B Gold</td>
<td>Selection antibiotic for the lph gene</td>
<td>ant-hg-1</td>
</tr>
<tr>
<td>Puromycin</td>
<td>Selection antibiotic for the pac gene</td>
<td>ant-pr-1</td>
</tr>
<tr>
<td><strong>Fast-Media® Zeo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast-Media® Zeo Agar</td>
<td>Solid agar selection medium containing Zeocin™</td>
<td>fas-zn-s</td>
</tr>
<tr>
<td>Fast-Media® Zeo TB</td>
<td>Liquid TB selection medium containing Zeocin™</td>
<td>fas-zn-l</td>
</tr>
<tr>
<td>Fast-Media® Zeo X-Gal</td>
<td>Solid agar selection medium containing Zeocin™ &amp; X-Gal</td>
<td>fas-zn-x</td>
</tr>
<tr>
<td><strong>Plasmids encoding the Sh ble gene</strong></td>
<td>Plasmid encoding a synthetic Sh ble gene</td>
<td>pmod2-zeo</td>
</tr>
<tr>
<td>pMOD2-Zeo</td>
<td>LacZ-expression plasmid selectable with Zeocin™</td>
<td>psetz-lacz</td>
</tr>
<tr>
<td>pSELECT-zeo-LacZ</td>
<td>Expression plasmid selectable with Zeocin™</td>
<td>psetz-mcs</td>
</tr>
</tbody>
</table>

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 Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com