invitrogen™
by life technologies™

# Zeocin™

| Cat. nos. | Size | Store at –30°C to –10°C |
|---|---|---|
| R250-01 | 1 g (8 × 1.25 mL) | |
| R250-05 | 5 g (50 mL) | |

**Pub. Part no.** R250.pps MAN0001537 **Rev. Date** 27 Jan 2012

## Description

Zeocin™ is a formulation of phleomycin D1, a basic, water-soluble, copper-chelated glycopeptide isolated from *Streptomyces verticillus* and shows strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cell lines. The blue color of the solution is due to the presence of copper and the copper-chelated form of Zeocin™ is inactive. When the antibiotic enters the cell, the copper cation is reduced from $Cu^{2+}$ to $Cu^{1+}$ and removed by sulfhydryl compounds in the cell. Upon copper removal, Zeocin™ is activated, and binds and cleaves DNA, causing cell death.

A Zeocin™ resistance protein of 13,665 Da, has been isolated and characterized. The protein is the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), binds stoichiometrically to Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

**Note:** Basic information on using Zeocin™ is described in this insert. For details including Zeocin™ structure and photographs of Zeocin™ treated cells, download the Zeocin™ manual from **www.lifetechnologies.com**.

## Specifications

| | |
|---|---|
| Contents: | 100 mg/mL solution in deionized, autoclaved water. |
| Shipping/Storage: | Shipped on blue ice. Store at –30°C to –10°C. |
| *E. coli* Selection: | 25–50 µg/mL in low salt LB medium*<br>*(NaCl concentration should not exceed 5 g/liter.) |
| Yeast Selection: | 50–300 µg/mL in YPD or minimal medium |
| Mammalian Cell Selection: | 50–1000 µg/mL in suitable medium (varies with cell line). |

**Intended Use:** For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

## Handling Zeocin™

- Always wear gloves, a laboratory coat, and safety glasses when handling Zeocin™ containing solutions.
- Zeocin™ is light sensitive. Store the antibiotic and plates or medium containing the antibiotic in the dark.
- Reduce the salt in bacterial medium and adjust the pH to 7.5 to keep Zeocin™ active as high ionic strength and acidity or alkalinity inhibit Zeocin™ activity.
- Store Zeocin™ at −30°C to −10°C and thaw on ice before use.

## Zeocin™ Selection in *E. coli*

**Host:** Must **not** contain the Tn5 transposon (*i.e.* TOP10, DH5, DH10).

**Medium:** Use Low Salt LB Medium (10 g Tryptone, **5 g NaCl**, and 5 g Yeast Extract) at pH 7.5 to prevent inactivation of Zeocin™.

**Selection:** Use 25–50 µg/ml of Zeocin™ for selection in *E. coli*.

## Zeocin™ Selection in Yeast

**Yeast:** *Saccharomyces cerevisiae*, *Pichia pastoris*

**Medium:** YPD with 1 M sorbitol (electroporated cells); YPD or minimal plates (chemically transformed cells). Test the medium adjusted to pH values ranging from 6.5–8.0 and select the pH that allows you to use lowest Zeocin™ concentration.

**Transformation Method:** Use electroporation, lithium cation protocols, or EasyComp™ Kits. **Do not** use spheroplasting for yeast transformation with Zeocin™ containing plasmids as it results in complete cell death.

**Selection:** Use 50–300 µg/ml of Zeocin™, depending on the yeast strain, and media pH and ionic strength. Perform a kill curve to determine the lowest Zeocin concentration required to kill the untransformed host strain.

**Note:** Allow the cells to recover for 1 hour in YPD medium after transformation. To obtain efficient Zeocin™ selection, plate at low cell densities (use 10, 25, 50, 100, and 200 µL of transformation reaction).

## Zeocin™ Selection in Mammalian Cells

Use 50–1000 µg/mL of Zeocin™ to select stable cell lines (the average is about 250–400 µg/mL). Depending on the cell line, it takes 2–6 weeks to generate foci with Zeocin™. Determine the minimum concentration required to kill the untransfected host cell line prior to generating stable cell lines (see below).

## Determining Zeocin™ Sensitivity

1. Plate or split a confluent plate to obtain cells at ~25% confluency. Prepare a set of 8 plates. Grow cells for 24 hours. Remove the medium.
2. Add medium with varying Zeocin™ concentrations (0, 50, 100, 200, 400, 600, 800, and 1000 µg/mL) to each plate.
3. Replenish selective medium every 3–4 days and observe the percentage of surviving cells. Select the concentration that kills the majority of cells within 1–2 weeks.

## Selecting Stable Integrants

1. Transfect your cell line and plate onto 100 mm culture plates. Include a sample of untransfected cells as a negative control.
2. After transfection, wash the cells once with 1X PBS and add fresh medium to the cells.
3. Forty-eight to 72 hours after transfection, split the cells into fresh medium containing Zeocin™ at the pre-determined concentration required for your cell line. To have a better chance at identifying and selecting foci, we recommend using different cell dilutions.
4. Feed the cells with selective medium every 3–4 days until cell foci are identified.
5. Pick and transfer colonies to 96- or 48-well plates. Grow cells to near confluence before expanding to larger wells or plates.

## Selection Tip

If your cells are more resistant to Zeocin™, split cells into medium containing Zeocin™ and incubate the cells at 37ºC for 2–3 hours to let cells attach. Place the cells at 4ºC for 2 hours. Remember to buffer the medium with HEPES. Return the cells to 37ºC. Incubating the cells at 4°C stops the cell division process for a short time, allowing Zeocin™ to act, resulting in cell death.

## Maintaining Stable Cell Lines

- Maintain cells in the same Zeocin™ concentration used for selection
- Reduce the Zeocin™ concentration to a concentration that just prevents growth of sensitive cells but does not kill them (refer to the kill curve experiment)

## Accessory Products

Media for bacteria and mammalian cells as well as transformation products (yeast and bacteria) and transfection reagents are available from Life Technologies. Visit **www.lifetechnologies.com/support** for details.

## Certificate of Analysis

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