Zeocin

V02

Cat: Z8020
Specification: 1.25mL/ 8 × 1.25 mL
Storage: Store at -20°C, and it is valid for 2 years.

Introduction

Zeocin is a formulation of phleomycin D1, a basic, water-soluble, copperchelated 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^{+} 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.

Specifications:

Contents:	100 mg/ml solution in deionized, autoclaved water.
Shipping/Storage:	Shipped on blue ice. Store at -20°C.
E. coli Selection:	25-50 μg/ml in low salt LB medium (NaCl concentration should not exceed 5 g/liter.)
Yeast Selection:	50-300 μg/ml in YPD or minimal medium
Mammalian Cells	50-1000 μg/ml in suitable medium (varies with cell Selection: line).

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 basicity inhibit Zeocin activity.
- Store Zeocin at -20°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 your 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 using various dilutions 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.

Zeocin Selection in Mammalian Cells, Continued

Selection Tip

If your cells are more resistant to Zeocin, split cells into medium containing Zeocin and incubate the cells at 37°C for 2-3 hours to let cells attach. Place the cells at 4°C for 2 hours. Remember to buffer the medium with HEPES. Return the cells to 37°C.

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 by half or to a concentration that just prevents growth of sensitive cells but does not kill them (refer to the kill curve experiment)

Product Qualification

Zeocin is lot qualified by demonstrating that LB media containing 35 μg/ml Zeocin prevents growth of the E. coli strain,TOP10