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Zeocin™ Selection Reagent

USER GUIDE

Catalog Numbers R250-01, R250-05

Publication Number MAN0000019

Revision A.0

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0000019

Revision	Date	Description
A.0	22June2017	Rebrand. Remove molecular weight from Zeocin™ figure.
19 January 2012		Baseline for this revision

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Product information

Product information

Zeocin™ Selection Reagent is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. It shows strong toxicity against bacteria, fungi (including yeast), plants and mammalian cell lines. Since Zeocin™ Selection Reagent is active in both bacteria and mammalian cell lines, vectors can be designed that carry only one drug resistance marker for selection.

Zeocin™ Selection Reagent is a formulation of phleomycin D1, a basic, water-soluble, copper-chelated glycopeptide isolated from *Streptomyces verticillus*. The presence of copper gives the solution its blue color. This copper-chelated form 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 removal of the copper, Zeocin™ is activated and will bind DNA and cleave it, causing cell death.

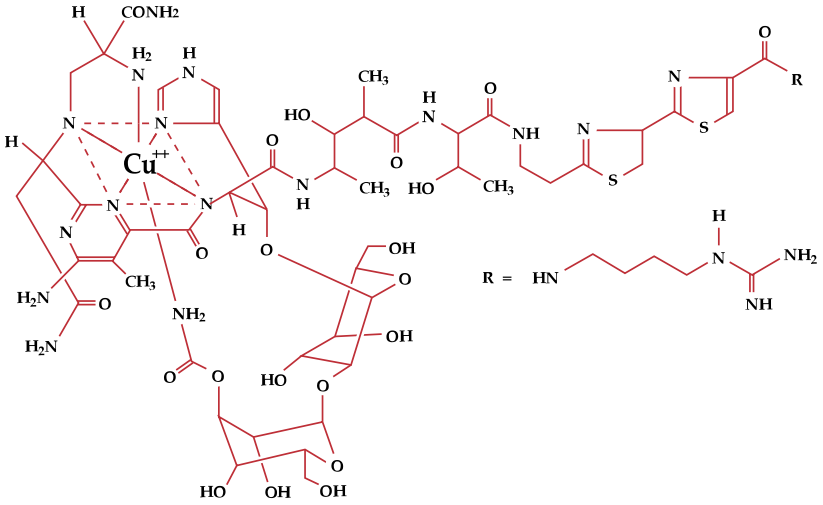

Figure 1 The structure of Zeocin™

Content and storage

Zeocin™ Selection Reagent is shipped on blue ice, and is supplied as a 100 mg/mL solution in deionized, autoclaved water

Contents	Cat. No.	Amount[1]	Storage
Zeocin™ Selection Reagent (1 g)	R250-01	8 × 1.25 mL	–20°C
Zeocin™ Selection Reagent (5 g)	R250-05	50 mL	

[1] 100 mg/mL solution in deionized, autoclaved water

Methods

Zeocin™ procedural guidelines

Resistance to Zeocin™

The Zeocin™ resistance protein is a 13,665 Da protein. It 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™.

Guidelines for handling Zeocin™

- High ionic strength and acidity or basicity inhibit the activity of Zeocin™. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see Low Salt LB Medium).
- Store Zeocin™ at –20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store the drug and plates or medium containing the drug in the dark.
- See Appendix A for general safety information.

Zeocin™ Selection Reagent concentration for selection

Organism	Zeocin™ Selection Reagent concentration
E. coli	25–50 µg/mL in low salt LB medium[1]
Yeast	50–300 µg/mL in YPD or minimal medium
Mammalian cells	50–1000 µg/mL (varies with cell line)

[1] For efficient selection, the concentration of NaCl should not exceed 5 g/liter.

Zeocin™ selection in *E. coli*

Procedural guidelines

- Use 25–50 µg/mL of Zeocin™ for selection in *E. coli*.
- High salt and extremes in pH will inhibit the activity of Zeocin™
- Any *E. coli* strain that contains the complete Tn5 transposable element (i.e. DH5αF′IQ, SURE, SURE2) encodes the *ble* (bleomycin) resistance gene. These strains will confer resistance to Zeocin™. For the most efficient selection, use an *E. coli* strain that does not contain the *Tn5* gene (i.e. TOP10, DH5, DH10, etc.).
- Extremes in pH and high ionic strength will inhibit the activity of Zeocin™. To optimize selection in *E. coli*, the salt concentration must be < 110 mM and the pH must be 7.5. A recipe for Low Salt LB is provided to optimize selection in *E. coli*.

Prepare Low Salt LB Medium

1. Combine:

Reagent	Amount
Tryptone	10 g
NaCl	5 g
Yeast extract	5 g
Distilled water	950 mL

   Adjust pH to 7.5 with 1 N NaOH and bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.

2. Autoclave on liquid cycle at 15 psi and 121°C for 20 minutes.

3. Thaw Zeocin™ on ice and vortex before removing an aliquot.

4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/mL final concentration.

5. Store plates and unused medium at +4°C in the dark. Plates and medium containing Zeocin™ are stable for 1–2 weeks.

Zeocin™ selection in yeast

Procedural guidelines

- The concentration of Zeocin™ required to select resistant transformants may range from 50 to 300 µg/mL, depending on the strain, pH, and ionic strength.
- **IMPORTANT!** We do not recommend spheroplasting for transformation of yeast with plasmids containing the Zeocin™ resistance marker. Spheroplasting involves removal of the cell wall to allow DNA to enter the cell. Cells must first regenerate the cell wall before they are able to express the Zeocin™ resistance gene. Plating spheroplasts directly onto selective medium containing Zeocin™ will result in complete cell death.

Guidelines for yeast transformation

- We recommend electroporation, lithium cation protocols, or our EasyComp™ Kits for transformation of yeast with vectors that encode resistance to Zeocin™.
- Electroporation yields 10^3 to 10^4 transformants per µg of linearized DNA and does not destroy the cell wall of yeast.
- If you do not have access to an electroporation device, use chemical methods or one of the EasyComp™ Kits listed:

Kit	Reactions	Cat. No.
S. c. EasyComp™ Transformation Kit (for Saccharomyces cerevisiae)	6 × 20 transformations	K5050-01
Pichia EasyComp™ Transformation Kit (for *Pichia pastoris*)	6 × 20 transformations	K1730-01

Guidelines for ionic strength and pH

- Since yeast vary in their susceptibility to Zeocin™, we recommend that you perform a kill curve to determine the lowest concentration of Zeocin™ needed to kill the untransformed host strain.
- The pH of the selection medium may affect the concentration of Zeocin™ needed to select resistant transformants.
- Test media adjusted to different pH values (6.5 to 8) for the one that allows you to use the lowest possible concentration of Zeocin™.

Guidelines for selection in yeast

- After transformation (either by electroporation or chemical transformation), allow the cells to recover for one hour in YPD medium.
- For electroporated cells, plate your transformants on YPD containing 1 M sorbitol. Sorbitol allows better recovery of the cells after electroporation.
- For chemically transformed cells, plate cells on YPD or minimal plates.
- Plate several different volumes (i.e. 10, 25, 50, 100, and 200 µL) of the transformation reaction. Plating at low cell densities favors efficient Zeocin™ selection.

Zeocin™ selection in mammalian cells

Mammalian cells exhibit a wide range of susceptibility to Zeocin™. Concentrations of Zeocin™ used to select stable cell lines may range from 50 to 1000 µg/mL, with the average being around 250 to 400 µg/mL. Factors that affect selection include ionic strength, cell line, cell density, and growth rate.

Mechanism of Zeocin™

The killing mechanism of Zeocin™ is quite different from neomycin and hygromycin. Cells do not round up and detach from the plate. Sensitive cells may exhibit the following morphological changes upon exposure to Zeocin™:

- Vast increase in size (similar to the effects of cytomegalovirus infecting permissive cells)
- Abnormal cell shape, including the appearance of long appendages
- Presence of large empty vesicles in the cytoplasm (breakdown of the endoplasmic reticulum and Golgi apparatus or scaffolding proteins)
- Breakdown of plasma and nuclear membrane (appearance of many holes in these membranes)

Eventually, these cells will completely break down and only cellular debris will remain.

Zeocin™-resistant cells should continue to divide at regular intervals to form distinct colonies. There should not be any distinct morphological changes in Zeocin™-resistant cells when compared to cells not under selection with Zeocin™.

For examples of HEK 293 and COS1 cell undergoing selection in the presence of Zeocin™, see Appendix A.

Procedural guidelines

Guidelines for ionic strength and pH

For selection in mammalian cells, physiological ionic strength and pH are much more important for cell growth, so more Zeocin™ may be needed for selection relative to yeast or bacteria.

Guidelines for selection in mammalian cells lines

To generate a stable cell line that expresses your protein from an expression construct, you need to determine the minimum concentration required to kill your untransfected host cell line. In general, it takes 2–6 weeks to generate foci with Zeocin™, depending on the cell line. Because individual cells can express protein at varying levels, it is important to isolate several foci to expand into stable cell lines.

Determine Zeocin™ sensitivity

Determine the minimal concentration of Zeocin™ required to kill the untransfected parental cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 8 plates. Grow cells for 24 hours.
2. Remove medium and then add medium with varying concentrations of Zeocin™ (0, 50, 100, 200, 400, 600, 800, and 1000 µg/mL) to each plate.
3. Replenish the selective medium every 3–4 days and observe the percentage of surviving cells over time.

   Select the concentration that kills the majority of the cells in the desired number of days (within 1–2 weeks).
4. If you have trouble distinguishing viable cells by observation, we recommend counting the number of viable cells by trypan blue exclusion to determine the appropriate concentration of Zeocin™ required to prevent growth.

Selection tip

Some cells may be more resistant to Zeocin™ than others. If cells are rapidly dividing, Zeocin™ may not be effective at low concentrations. We suggest trying the following protocol to overcome this resistance:

1. Split cells into medium containing Zeocin™.
2. Incubate cells at 37°C for 2–3 hours until the cells have attached to the culture dish.
3. Remove the plates from the incubator and place the cells at +4°C for 2 hours. Be sure to buffer the medium with HEPES.
4. Return the cells to 37°C.

   Incubating the cells at +4°C will stop the cell division process for a short time, allow Zeocin™ to act, and result in cell death.

Select stable integrants

Once you have determined the appropriate Zeocin™ concentration to use for selection, you can generate a stable cell line with your construct.

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. By using different dilutions, you will have a better chance at identifying and selecting foci. .

   Note: If your cells are more resistant to Zeocin™, you may want to use the selection tip described on the previous page. Simply split cells into medium containing Zeocin™, incubate at 37°C for 2–3 hours to let cells attach, then place the cells at +4°C for 2 hours. Remember to buffer the medium with HEPES

4. Feed the cells with selective medium every 3–4 days until cell foci are identified.

5. Pick and transfer colonies to either 96- or 48-well plates. Grow cells to near confluence before expanding to larger wells or plates.

Maintain stable cell lines

To maintain stable cell lines, you may:

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

A

Cells under Zeocin™ selection

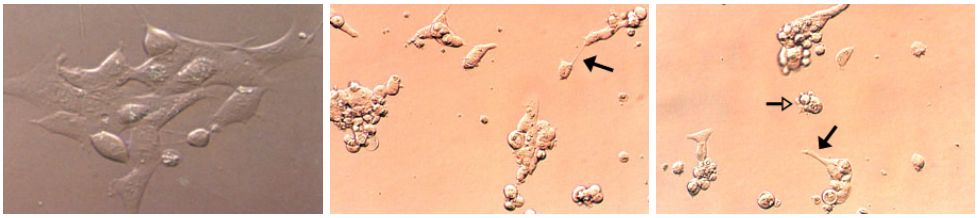

Figure 2 HEK 293 cells under Zeocin™ selection

HEK 293 cells undergoing Zeocin™ selection. Cells were cultured in DMEM containing 10% FBS, 1 mM L-glutamine, and 400 µg/mL Penicillin-Streptomycin in the absence or presence of 400 µg/mL Zeocin™. Left) Unselected cells. Center) Zeocin™-sensitive cells. Long appendages may appear to grow out from the cell as the plasma membrane breaks down. Right) Zeocin™-sensitive cells. Cells will begin to disintegrate and cell particles may be observed in the medium.

Figure 3 COS1 cells under Zeocin™ selection

COS1 cells undergoing Zeocin™ selection. Cells were cultured in DMEM containing 10% FBS, 1 mM L-glutamine, and 400 µg/mL Penicillin-Streptomycin in the absence or presence of 400 µg/mL. Zeocin™ Left) COS1 cells not exposed to Zeocin™. Center)Zeocin™ sensitive cells. Cells will begin to disintegrate and cell particles may be observed in the medium. Right) Zeocin™-sensitive cells. Long appendages may appear to grow out from thecell as the plasma membrane breaks down

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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