USER GUIDE

invitrogen™
by life technologies™

Zeocin™ Selection Reagent

Catalog nos. R250-01, R250-05

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life technologies™

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Important Information

Shipping and Storage

Zeocin™ Selection Reagent is shipped on blue ice, and is supplied as a 100 mg/mL solution in deionized, autoclaved water. **Store at –20°C.**

Catalog No.	Amount	How Supplied
R250-01	1 g	8 x 1.25 mL
R250-05	5 g	50 mL

Intended Use

For research use only. Not intended for any human or animal diagnostic or therapeutic uses.

Methods

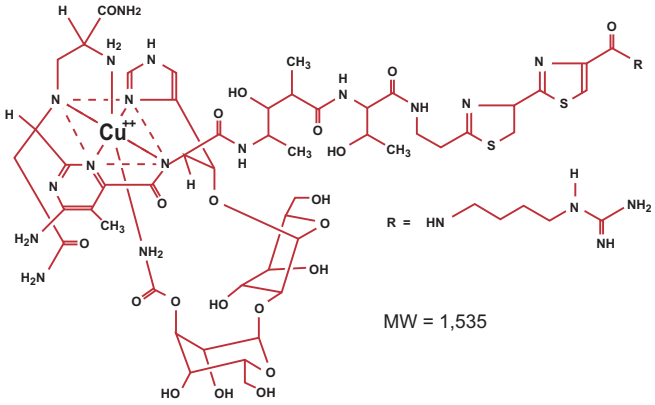
Overview

Introduction

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 (Calmels *et al.*, 1991; Drocourt *et al.*, 1990; Gatignol *et al.*, 1987; Mulsant *et al.*, 1988; Perez *et al.*, 1989). 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.

Description

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. The structure of Zeocin™ is shown below (Berdy, 1980).



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Overview, continued

Resistance to Zeocin™

A Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This 13,665 Da protein, 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™.

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**, page 4).
- 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.
- Wear gloves, a laboratory coat, and safety glasses when handling Zeocin™ containing solutions.
- Do not ingest or inhale solutions containing the drug.
- Be sure to bandage any cuts on your fingers to avoid exposure to the drug.

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Concentrations of Zeocin™ to Use for Selection

Zeocin™ and the *Sh ble* gene can be used for selection in mammalian cells (Drocourt *et al.*, 1990; Mulsant *et al.*, 1988), plants (Perez *et al.*, 1989), yeast (Calmels *et al.*, 1991; Gatignol *et al.*, 1987), and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ to use for selection in mammalian tissue culture cells, yeast, and *E. coli* are listed below.

Organism	Zeocin™ Concentration and Selective Medium
E. coli	25–50 µg/mL in Low Salt LB medium*
Yeast	50–300 µg/mL in YPD or minimal medium
Mammalian cells	50–1000 µg/mL (varies with cell line)

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

Zeocin™ Selection in *E. coli*

Introduction

Use 25–50 µg/mL of Zeocin™ for selection in *E. coli*. High salt and extremes in pH will inhibit the activity of Zeocin™ (see recommendations below).

E. coli Host

Any *E. coli* strain that contains the complete Tn*5* 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 Tn*5* gene (*i.e.* TOP10, DH5, DH10, etc.).

Ionic Strength and pH

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 below to optimize selection in *E. coli*.

Low Salt LB Medium

10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 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.

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

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Zeocin™ Selection in *E. coli*, continued

ImMedia™ growth medium is available from Life Technologies for fast and easy microwaveable preparation of Low Salt LB medium or agar containing Zeocin™. See below for ordering information. For more information, see our website (**www.lifetechnologies.com**) or call Technical Support (see page 14).

Medium	Quantity	Catalog no.
imMedia™ Zeo Liquid	20 pouches†	Q620-20
imMedia™ Zeo Agar	20 pouches†	Q621-20

†Each pouch provides sufficient reagents to prepare 200 mL of liquid medium or 8–10 standard size agar plates.

Zeocin™ Selection in Yeast

Introduction

We have successfully transformed plasmids conferring Zeocin™ resistance into *Saccharomyces cerevisiae* and *Pichia pastoris*. 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. Guidelines are provided below to assist you with selecting Zeocin™-resistant transformants.

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.

Transformation Method

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 below.

Kit	Reactions	Catalog 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

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Zeocin™ Selection in Yeast, continued

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. In addition, the pH of the selection medium may affect the concentration of Zeocin™ needed to select resistant transformants. We recommend that you 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™.

Selection in Yeast

For successful selection of Zeocin™-resistant transformants we recommend the following:

- 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

Introduction

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. Review the guidelines below to ensure successful selection of stable cell lines.

Important

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™.

Examples

To see photographs of HEK 293 and COS1 cells undergoing selection in the presence of Zeocin™, refer to the **Appendix**, pages 12 and 13.

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Zeocin™ Selection in Mammalian Cells, continued

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.

Selection in Mammalian Cell 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 (see **Determination of Zeocin™ Sensitivity**, below). 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.

Determining Zeocin™ Sensitivity

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

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).

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.

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Zeocin™ Selection in Mammalian Cells, continued

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.

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Zeocin™ Selection in Mammalian Cells, continued

Selecting 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.

Maintaining 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)

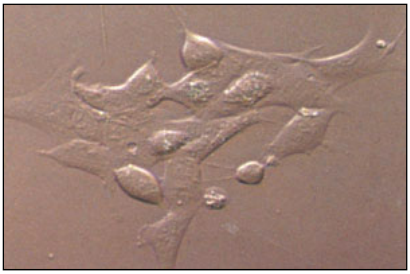
Appendix

HEK 293 Cells Under Zeocin™ Selection

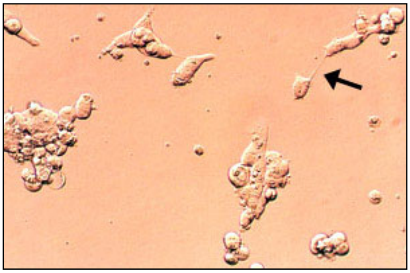
Introduction

The photographs below show HEK 293 cells (Graham *et al.*, 1977) 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™.

Panel A: 293 cells not exposed to Zeocin™
Panels B and C: 293 cells after 3 days in selective medium

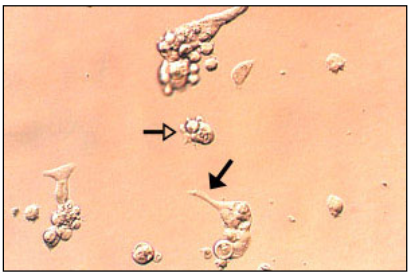


A. Unselected cells



B. Zeocin™-sensitive cells

Long appendages may appear to grow out from the cell as the plasma membrane breaks down (see filled arrow in this panel and below).



C. Zeocin™-sensitive cells

Cells will begin to disintegrate and cell particles may be observed in the medium (see open arrow in this panel).

COS Cells Under Zeocin™ Selection

Introduction

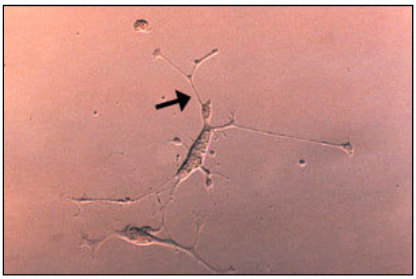
The photographs below show COS1 cells undergoing Zeocin™ selection. Cells were cultured in DMEM containing 10% FBS, 1 mM L-glutamine, and 400 µg/mL Penicillin-Streptomycin in the absence or presence of 400 µg/mL Zeocin™.

Panel A: COS1 cells not exposed to Zeocin™
Panels B and C: COS1 cells after 3 days in selective medium

Unselected cells

Zeocin™-sensitive cells

Cells will begin to disintegrate and cell particles may be observed in the medium (see filled arrow in this panel).



Zeocin™-sensitive cells

Long appendages may appear to grow out from the cell as the plasma membrane breaks down (see filled arrow in this panel).

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Sata Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

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Technical Support, continued

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Notes

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