MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Zeocin,100mg/ml

Product Number: BC-ZEC01-1ml

Shipping and Storage

Store at -20°C, valid for at least one year. Avoid repeated freeze-thaw cycles.

Component

Component	BC-ZEC01-1ml
Zeocin,100mg/mL	1mL

Description

Zeocin, with phleomycin D1 as the main active ingredient, is also known as bleomycin, bleomycin hydrochloride or phleomycin D1. It is a member of the bleomycin/phleomycin antibiotic family isolated from a mutant strain of Streptomyces verticillus. It exerts inhibitory and cytotoxic effects on bacteria, fungi, plants and mammalian cells, and is commonly used for the selection of polyclonal or monoclonal cells expressing the Zeocin resistance gene, or for the maintenance culture of corresponding polyclonal or monoclonal cells. The recommended concentration for E. coli selection is 25~50μg/mL (low-salt LB medium, NaCl concentration shall not exceed 5g/L); the recommended concentration for yeast selection is 50~300μg/mL; the recommended concentration for mammalian cell selection is 50~1000μg/mL (the average commonly used concentration is 250-400μg/mL).

This product is formulated in HEPES solution at a concentration of 100mg/mL. It is sterilized by membrane filtration and can be directly used for cell culture.

Protocol

This product is usually used at a concentration of 100μg/mL, which is a 1000-fold dilution from the stock solution. However, the main factors affecting the selection concentration include ionic strength, cell type, cell growth density and growth rate. Depending on the cell type, it takes 1-2 weeks to select Zeocin-resistant cells, and the optimal working concentration needs to be determined via a dose-response curve. The recommended concentrations for some mammalian cells are listed below.

Cell line	Medium	Concentration
B16 (Mouse melanocytes)	RPMI	20-250μg/mL
CHO (Chinese hamster ovarian cells)	DMEM	100-500μg/mL
COS (Monkey kidney cells)	DMEM	100-400μg/mL
HEK293 (Human embryonic kidney cells)	DMEM	100-400μg/mL
HeLa (Human uterine cells)	DMEM	50-100μg/mL
J558L (Mouse melanocytes)	RPMI	400μg/mL
MCF-7 (Human breast adenocarcinoma cells)	DMEM	100-400μg/mL
MEFs (Mouse embryonic fibroblasts)	DMEM	200-400μg/mL
THP-1 (Human monocytes)	RMPI	200μg/mL

1. Determination of Optimal Working Concentration

1.1. Seed cells at a confluency of approximately 25%. Prepare cells in 16 or 24 culture wells (for duplicate or triplicate wells respectively) and incubate for 24 h.

1.2. Remove the cell culture medium and replace with fresh selection medium containing gradient concentrations of Zeocin (0, 50, 100, 200, 400, 600, 800 and 1000μg/mL).

1.3. Refresh the selection medium every 2-4 days. Observe the proportion of viable cells, and select the lowest concentration that kills all cells within 1-2 weeks as the optimal working concentration.

2. Selection of Stable Cell Lines

2.1. Seed cells at a confluency of 20%-30% and incubate overnight.

2.2. Transfect cells with plasmids carrying the Zeocin resistance gene, or infect cells with viruses carrying the Zeocin resistance gene. Meanwhile, set untransfected/uninfected cells as the control group.

2.3. At 48-72 h after transfection or infection, replace with fresh medium containing the optimal working concentration of Zeocin and continue incubation. If necessary, cells can be passaged and slightly diluted for selection culture.

2.4. Refresh the Zeocin-containing selection medium every 2-4 days.

2.5. When all normal cells in the control group are completely dead, the surviving cells in the Zeocin-resistant group are cells expressing the Zeocin resistance gene. Polyclonal or monoclonal cell selection can then be performed according to experimental purposes.

3. Maintenance of Stable Cell Lines (One of the following methods can be selected)

3.1. Maintain the culture using Zeocin selection medium at the same concentration as that used for stable cell line selection above.

3.2. Reduce the Zeocin concentration to half of the selection concentration for maintenance culture.

3.3. Maintain the culture using a Zeocin concentration that just inhibits the growth of sensitive cells but is not lethal.

Note

1. This product is harmful to the human body. Please operate with caution and take effective protective measures to avoid direct contact with skin or inhalation.
2. This product is unstable under excessively high or low pH conditions and in the presence of weak oxidants, and such denaturation is irreversible. When culturing bacteria, appropriately reduce the salt concentration of the bacterial medium (low-salt LB medium, NaCl concentration shall not exceed 5 g/L) and adjust pH to 7.5 to maintain its activity.
3. This product is for scientific research use by professionals only. It shall not be used for clinical diagnosis or treatment, nor for food or pharmaceutical purposes.
4. For your safety and health, please wear a lab coat and disposable gloves during operation.