Hygromycin B

Solution

Cat. No. 10 843 555 001 1 g (20 ml)

Version 20
Content version: July 2019

Store the kit at +2 to +8°C

1. What this Product Does

Formulation

50 mg/ml in PBS (phosphate buffered saline), filtered through a 0.2 μm pore-size membrane.

⚠ R22: Harmful if swallowed.
S24/25: Avoid contact with skin and eyes.

Molecular Weight

527.5

Formula

$C_{20}H_{37}N_3O_{13}$

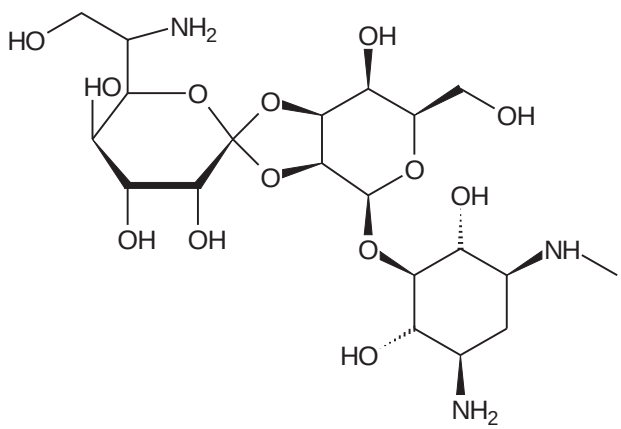

Fig. 1: Formula

CAS No.

31282-04-9

Purity

>80% (HPLC)

Storage and Stability

The solution is stable at +2 to +8°C until the expiration date printed on the label.

Working Concentration

Recommended concentration for the selection of resistant cells is 50 – 1,000 μg/ml (see Table 1). The optimal concentration must be tested experimentally and may vary with the cell type.

A commonly used concentration for selection of mammalian cells is 200 μg/ml.

Application

Hygromycin B is used for the selection of pro- and eukaryotic cells, stably transfected with the hygromycin resistance gene, as well as for the maintenance of the hygromycin phenotype of resistant cells.

2. How to Use this Product

2.1 Before You Begin

Overview

Cells can escape selection if the antibiotic is used at too low concentrations or if the plating density is too high.

The sensitivity of non-resistant cells also depends on the proliferative activity of the cells. Cells rapidly proliferating are killed faster than those, only slowly proliferating. Ideally, control cells should die within one week after addition of the antibiotic, allowing colonies of resistant cells to form within 10–14 days.

Determination of Toxic Concentration

Hygromycin B is added to the culture medium at a concentration that varies with the cell type transfected. A titration experiment for each cell type may therefore be performed to determine the amount of Hygromycin B necessary to kill untransfected cells.

A range between 50 μg/ml and 1 mg/ml should be tested.

Additional Media Required

Culture medium: The use of a chemically defined or serum-containing medium without addition of antibiotics, optimally meeting the specific media needs of the particular cell type or cell line to be cultured, is recommended.

2.2 Procedure

The procedure given below is a guideline that may be modified according to the respective test system.

🔍 For selection of transfected Hygromycin B resistant cells, a concentration of Hygromycin B should be used that completely blocks growth of sensitive, non-transfected cells.

❶	Plate non-transfected cells to be tested at a concentration of 50–200 cells/well in 200 μl culture medium containing various amounts of Hygromycin B (*e.g.,* 50–1,000 μg/ml) into microplates (tissue culture grade, 96-wells).
❷	Incubate cell cultures for 10–14 days.
❸	After 5–7 days, replace culture medium with fresh culture medium containing the respective amounts of Hygromycin B, if required. 🔍 A replacement of culture media containing Hygromycin B after 5–7 days is only necessary if nutritional compounds are supposed to be depleted by the cells cultured. A sign for such a depletion is the acidification of the culture medium. In this case, phenol red, a constituent of most media formulations, turns yellow.
❹	Evaluate cellular viability after 10–14 days using, for example, the Cell Proliferation Kit I (MTT)*, the Cell Proliferation Kit II (XTT)*, or Cell Proliferation Reagent WST-1*. Alternatively, 200–500 cells may be plated in 1–2 ml culture medium containing Hygromycin B as described into petri dishes (tissue culture grade, 35 mm) and incubated for 10–14 days. The cytotoxic effect may be determined by evaluating the number of surviving cell colonies or percent confluency.

2.3 Selection of Transfected Cells

Introduction

Selection of cells transfected with a DNA construct encoding for hygromycin resistance is performed, using culture medium containing Hygromycin B in a concentration determined for the particular experimental setup as described above (Section 2.2).

Additional Media Required

Prepare culture medium containing Hygromycin B as determined with non-transfected cells as described above (Section 2.2).

Procedure

1. Remove culture medium and add 5-6 ml fresh culture medium (containing Hygromycin B) to a 60 mm culture dish containing the freshly transfected cells.
   Suspension cells are centrifuged (10 minutes, 250 × *g*) using a sterile centrifugation tube prior to removal of medium and resuspended in approximately 5 ml culture medium containing Hygromycin B.
2. After 5–7 days, replace medium with fresh culture medium containing Hygromycin B as described, if required.
3. Incubate cells for an additional 5–7 days.
4. After this incubation period, the cell cultures will contain only living cells expressing the hygromycin B resistant phenotype. Therefore, culture medium containing Hygromycin B may be replaced with fresh culture medium as described above, but without addition of Hygromycin B.

Subculture of Adherent Cells

Depending on transfection efficiency and proliferation kinetics of the cells being transfected, it may be necessary to subculture adherent cells. This is of particular importance with adherent cells, since cells killed by Hygromycin B do not necessarily detach from the culture substrate.

1. After a subcultivation step, only viable cells will adhere to the culture substrate. This facilitates the evaluation of the cultures and optimizes the culture conditions for the surviving cells.
   - When Trypsin is used, subcultivation may be performed without changing the culture dish, by carefully removing the trypsin solution using a fine-tipped pasteur pipette, carefully leaving the cells behind.
2. Resuspend cells in 6 ml culture medium containing Hygromycin B and serum or a trypsin inhibitor.
3. Incubate cells in the same culture dish. For suspension cells, a dilution of cells may be necessary.

Replacement of Media

A replacement of culture media containing Hygromycin B after 5–7 days is only necessary if nutritional compounds are supposed to be depleted by the cells cultured. A sign of such depletion is the acidification of the culture medium. In this case, phenol red, a constituent of most media formulations, turns yellow.

To continue the selection process for a longer period, continue cultivation of cells in culture medium containing Hygromycin B, as described.

Maintenance of Hygromycin Resistant Phenotype

For maintenance of the hygromycin resistant phenotype of established transfected cell lines and for elimination of revertants, cells may be regularly cultured in culture medium containing Hygromycin B at the same concentration used for the initial selection.

Alternatively, the occurrence of revertants may be avoided by permanently culturing the cells in Hygromycin B containing culture medium. In the latter case, from the dose response curve as determined above (Section 2.1), a subtonic Hygromycin B concentration may be chosen.

2.4 Cloning of Transfected Cells

Overview

After successful transfection and selection, Hygromycin B resistant transfectants may be cloned. Single-cell cloning ensures that Hygromycin B resistant transfectants are derived from the same parental cell. Several methods for single-cell cloning, for example, by limiting-dilution or by picking individual cell colonies, may be employed.

For cloning, single-cell suspensions are to be prepared and cells are plated at low densities. Therefore, adherent cells are to be subcultured. Even though every attempt is made to ensure that the cells are in single-cell suspension prior to plating, it is not guaranteed that colonies do not arise from two cells sticking together.

Therefore, cloning should be done at least twice ("recloning") to generate a clonal population.

Additional Medium Required

The media formulations used are the same as for the standard culture of the respective cell type or for the selection procedure (2). Whether or not Hygromycin B is added is to be decided according to the particular experimental setup, as described above.

Cloning by Limiting Dilution

In the following table, cloning by limiting dilution is described.

- The transfectants should be healthy and rapidly proliferating at the time of cloning.

1. Plate cells into a multi-well culture plate (tissue culture grade, 96- or 24-wells, according to the particular application) such that approximately 1 cell will be plated per well in a final volume of 200 ml culture medium for 96-well plates or 1 ml culture medium for 24-well plates, respectively.
2. Incubate cultures according to their respective requirements.
   - Clones will appear within several days and should be subcultured or passaged according to the particular needs of the cell type cultured, for example, when reaching confluency.
3. Prior to dilution and plating steps, cell suspensions are to be carefully resuspended.

Cloning by Picking

In the following table, cloning by picking is described.

1. Plate adherent cells into petri dishes (60 mm or 100 mm, tissue culture grade) at a density of approximately 5×10^3 cells for a 60 mm petri dish or $1–1.5 \times 10^4$ cells for a 100 mm petri dish, respectively.
2. After several days, colonies of cells (3–10 cells) will appear.
3. Incubate cultures according to their respective needs.
4. After several days, colonies of cells (3–10 cells) will appear.
5. With the aid of an inverted microscope, the tip of a fire-polished Pasteur pipette is placed adjacent to the selected colony, and cells from the colony are "picked" by suction.
6. Picked cells are transferred into fresh culture dishes and subsequently cultured according to their respective requirements.
   - When cloning by picking is to be performed with suspension cells, plating of cells in soft agar is recommended.

3. Additional Information on this Product

3.1 How this Product Works

Action

Hygromycin B is an aminoglycosidic antibiotic produced by *Streptomyces hygroscopicus* that kills bacteria, fungi, and higher eukaryotic cells by inhibiting protein synthesis. It has been reported to interfere with translocation (1, 2) and to cause mistranslation (3).

Resistance

A gene has been identified that confers resistance in E. coli against Hygromycin B. The resistance gene codes for kinase (hygromycin B phosphotransferase) that inactivates Hygromycin B through phosphorylation.

Cloning of the resistance gene (designated HM+, HMR, hyg, or hph) and fusion with eukaryotic promoters has resulted in the construction of vectors that allow selection for resistance to Hygromycin B in both prokaryotic and eukaryotic cell systems. A variety of vectors have been developed (4–13). Hygromycin B has been used for selection of a wide variety of transfected cells (see Table 1) (4-23).

Table 1

Concentration of hygromycin B used for selection of cells after transfection with DNA constructs encoding for hygromycin resistance as taken from the literature.

Species	Cell Type	Cell Line/ Strain	Reference	Hygromycin B (μg/ml)
E. coli		JM 83	13 5	25–100 200
Streptomyces lividans			4	50
Saccharomyces cerevisiae			5	200
Aspergillus nidulans		GR 1 GR 5 G 191 GB 20 W 1 FGSC 4 FGSC 237	15 15 15 15 15 15 15	250 250 250 750 >1,000 >1,000 >1,000
Tobacco			13, 20	20–200
Chicken		DT 40 27 C 2 RP 9 HD 3 CU 39 BM 2	18 18 18 18 18 18	1,500 1,500 1,500 1,500 1,500 1,500
Mouse	ES cells	LTK L 929 C 127 NIH 3T3 PA 317 ψ 2 Ω E	6, 7, 9 23 10 11, 12 11 11 11 19	200–400 200 300 50–500 50 150 50 120
Rat	Embryo fibroblasts		16	200
Mink		CCL 64	7	400
Human		B 95–8 Raji 721 WIL 2 TK-/- Daudi GG 68 293 K-562	8 8 8 8 8 8 14 21	50 100 100 200 200 400 200 200
Mouse/human	Hybrid cell	SCC 16-5	23	200

G-418 Solution

G-418 Solution* is another type of aminoglycoside antibiotic commonly used for selection of transfected eukaryotic cells.

This antibiotic can be inactivated by the bacterial aminoglycoside phosphotransferases APH(3')II and APH(3')I encoded by genes on transposons Tn5 and Tn601 (also known as Tn903), respectively. Transfection of the neomycin resistance gene(s) (neo) from transposon Tn5 or Tn601 into cells results in resistance to G-418 Solution (neo r) and enables the cells to grow in media containing G-418 Solution (24–28). Resistance to neomycin and to hygromycin can be selected for independently and simultaneously in cell lines that have been transfected with both genes. Thus, two different vectors can be introduced into one cell line, either simultaneously or sequentially.

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4. Supplementary Information

Conventions

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Text Conventions

To make information consistent and understandable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Changes to Previous Version

Editorial changes.

Ordering Information

Product	Pack Size	Cat. No.
Apoptosis and Cell Death Products		
Cell Proliferation Reagent WST-1	25 ml (2,500 tests) 8 ml (800 tests)	11 644 807 001 05 015 944 001
Cytotoxicity Detection Kit^{PLUS} (LDH)	1 kit 400 tests in 96 wells 1 kit 2,000 tests in 96 wells	04 744 926 001 04 744 934 001
Gene Knockdown Reagent		
X-tremeGENE siRNA Transfection Reagent	1 ml (400 transfections in a 24-well plate)	04 476 093 001
Mycoplasma Detection Reagents		
Mycoplasma Detection Kit	1 kit (25 tests)	11 296 744 001
Mycoplasma PCR ELISA	1 kit (96 reactions)	11 663 925 910
Plasmid Isolation Products		
Genopure Plasmid Midi Kit	1 kit (for up to 20 preparations)	03 143 414 001
Genopure Plasmid Maxi Kit	1 kit (for up to 10 preparations)	03 143 422 001
Protease Inhibitor Tablets and Lysis Reagents		
cOmplete	20 tablets in glass vials 3 × 20 tablets in glass vials 20 tablets in *EASYpacks*	11 697 498 001 11 836 145 001 04 693 116 001
cOmplete, EDTA-free	20 tablets in a glass vial 3 × 20 tablets in glass vials 20 tablets in *EASYpacks*	11 873 580 001 05 056 489 001 04 693 132 001
cOmplete Lysis-M (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 complete Protease Inhibitor Cocktail Tablets)	04 719 956 001
cOmplete Lysis-M,EDTA-free (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 complete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 964 001
Reporter Gene Assays		
CAT ELISA	1 kit (192 tests)	11 363 727 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit (500 assays, microplate format, 250 assays, tube format)	11 758 241 001
β-Gal ELISA	1 kit (192 tests)	11 539 426 001
hGH ELISA	1 kit (192 tests)	11 585 878 001
Luciferase Reporter Gene Assay, high sensitivity	200 assays 1,000 assays	11 669 893 001 11 814 036 001
SEAP Reporter Gene Assay, chemiluminescent	1 kit (500 assays, microplate format, or 250 assays, tube format)	11 779 842 001
Selection Antibiotics		
G-418 Solution	20 ml 100 ml	04 727 878 001 04 727 894 001
Transfection Reagents		
X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1 ml 5 × 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
X-tremeGENE HP DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 366 244 001 06 366 236 001 06 366 546 001
Western Blotting Reagents		
Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1 kit (1,000 cm^2 membrane)	12 015 218 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml (1,000 cm^2 membrane)	12 015 196 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3.00 m)	03 010 040 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm^2) 6 × 100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
Cellular Analysis		
RTCA Analyzer		05 228 972 001
RTCA SP Station		05 229 057 001
RTCA MP Station		05 331 625 001
RTCA Control Unit 1.1		05 454 417 001
E-Plate 96	6 Units 6 × 6 Units	05 232 368 001 05 232 376 001
E-Plate VIEW 96	6 Units 6 × 6 Units	06 472 451 001 06 472 460 001
Cellavista Basic Magnification: 4×, 10× Illumination: Brightfield only		05 651 522 001
Cellavista Medium Magnification: 4×, 10×, 20× Illumination: Brightfield and Fluorescence, UV, Blue, Green		05 651 549 001
Cellavista High End Magnification: 2×, 4×, 10×, 20×, 40× Illumination: Brightfield and Fluorescence, UV, Blue, Cyan, Green, Amber, Red		05 651 557 001

Product	Pack Size	Cat. No.
Cedex XS Analyzer with Control Unit		05 926 432 001
Cedex Smart Slide package	15 × 8 measurements	05 650 801 001
CASY Model TT 45, 60, 150 µm		05 651 735 001

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