For life science research only.
Not for use in diagnostic procedures.

G-418 Solution

Version: 06
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Antibiotic solution for selection of neomycin resistance in transfected cells

Cat. No. 04 727 878 001	20 ml
	1 g
Cat. No. 04 727 894 001	100 ml
	5 x 20 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	G-418 Solution, 50 mg/ml	Filtered through a 0.2 µM pore-size membrane.	04 727 878 001	1 vial, 1 g, 20 ml
			04 727 894 001	5 vials, 5 g, 20 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	G-418 Solution	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Microplates, tissue-culture grade
- Petri dishes, tissue-culture grade

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.

For Cell Viability

- Cell Proliferation Kit I (MTT)*
- Cell Proliferation Kit II (XTT)*
- Cell Proliferation Reagent WST-1*

For Transfection

- DOTAP Liposomal Transfection Reagent*

For Subculturing Cells

- Dispase*

1.4. Application

G-418 Solution is used for:
- The selection of eukaryotic cells that are stably transfected with the neomycin-resistance genes (neo),
- as well as for the maintenance of the phenotype (neo^r) of resistant cells.
- The elimination of contaminating fibroblasts from mixed cultures.

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Determination of G-418 Concentration

1. In microplates (tissue-culture grade, 96 wells), plate 50 to 200 cells/well of non-transfected cells in 200 µl culture medium containing various amounts of G-418.

   ⚠ ***Recommended G-418 concentration for the selection of neo-resistant (neo^r) cells is 50 µg to 1 mg/ml.***

2. If necessary, after 5 to 7 days, replace the culture medium in each well with fresh culture medium containing the same amount of G-418.

   ℹ *Culture media containing G-418 needs to be replaced after 5 to 7 days only if nutritional compounds in the original media are depleted by the cultured cells. An indication of such depletion is acidification of the culture medium. In this case, phenol red, which is common in most media formulations, turns yellow.*

   - Incubate cell cultures for 10 to 14 days.

3. Evaluate cellular viability after 10 to 14 days using, for example, the Cell Proliferation Kit I (MTT])*, Cell Proliferation Kit II (XTT)*, or the Cell Proliferation Reagent WST-1*.

4. Choose a concentration of G-418 that completely blocks growth of sensitive, non-transfected cells.
   - Use this concentration in all transfection experiments to select for resistant (neo^r) cells.

Alternative Protocol for Determination of G-418 Concentration

1. Plate 200 to 500 cells in 1 to 2 ml culture medium containing G-418 on Petri dishes (tissue-culture grade, 35 mm diameter).

2. Incubate for 10 to 14 days.

3. Determine the cytotoxic effect either by evaluating the number of surviving cell colonies or percent confluency.

Transfection with DOTAP Liposomal Transfection Reagent*

For transfection of cells and subsequent selection of neomycin-resistant (neo^r) cells, use either:
- A plasmid construct that contains both the neomycin-resistance gene and the gene to be expressed, or
- A 1:10 mixture of two plasmids (*i.e.*, 1 part of a vector carrying the neomycin-resistance gene and 10 parts of a separate vector carrying the gene to be expressed).

⚠ ***The latter technique may lead to the survival of cells that only express the resistant (neo^r) phenotype but not the gene of interest. It also may reduce the yield of positive clones.***

ⓘ *Transfection of eukaryotic cells with reagents such as DOTAP Liposomal Transfection Reagent relies on the spontaneous formation of complexes between the cationic transfection reagent and nucleic acids. The complexes then fuse with the cell membrane and release the nucleic acids into the cytoplasm. These transfection reagents can introduce nucleic acid into eukaryotic cells with high efficiency in either serum-containing or serum-free media. They also have minimal cytotoxic effects. For a detailed description of the transfection protocol, see the Instructions for Use of the DOTAP Liposomal Transfection Reagent.*

Selecting Transfected Cells

The following protocol shows how to use culture medium containing G-418 to select cells that have been transfected with a DNA construct that conveys neomycin resistance (neo^r).

⚠ ***To determine the correct concentration of G-418 to use in this protocol, follow the steps described in section, Determination of G-418 Concentration.***

Protocol for Suspension Cells

1. Prepare culture medium containing G-418 at a suitable concentration determined with non-transfected cells, see section **Determination of G-418 Concentration**.

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2. In a sterile centrifugation tube, pellet the cell suspension (10 minutes at 250 × *g*).
   - Discard the supernatant.
   - For a 60 mm culture dish, resuspend cells in approximately 5 ml culture medium containing G-418.

   ⚠ ***Be sure to pellet and resuspend the cells in a sterile centrifugation tube. You may need to dilute the cell suspension for best results.***

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3. If necessary, after 5 to 7 days replace medium with fresh culture medium containing the same amount of G-418.

   ⓘ *Culture media containing G-418 needs to be replaced after 5 to 7 days only if nutritional compounds in the original media are depleted by the cultured cells. An indication of such depletion is acidification of the culture medium. In this case phenol red, which is common in most media formulations, turns yellow.*

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4. Incubate cells for an additional 5 to 7 days.

   ⓘ *In some cases, the selection process may need to be continued for longer periods. If your cell system requires longer incubation periods, maintain the cells in the same concentration of G-418 throughout the selection process.*

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5. After the 10 to 14 day selection period, the only living cells in the cultures will be those that express the resistant (neo^r) phenotype.
   - Therefore, at the end of the selection period, you may replace the culture medium containing G-418 with fresh culture medium that does not contain G-418.

⚠ ***To maintain the resistant (neo^r) phenotype of established transfected cell lines and to eliminate revertants, you should periodically grow the cells in culture medium that contains the same concentration of G-418 used for this initial selection process. Alternatively, you may avoid the occurance of revertants by permanently culturing the cells in culture medium containing lower concentrations of G-418. In this latter case, use the dose response curve (see section, Determination of G-418 Concentration) to choose the appropriate lower, subtoxic concentration.***

Modifications of Selection Protocol for Adherent Cells

Follow the **Protocol for Suspension Cells** with the following exceptions:

1. Omit the centrifugation in Step 2.

2. Depending on the transfection efficiency and proliferation kinetics of the cells being transfected, it may be necessary to subculture adherent cells (*e.g.*, using trypsin or Dispase*) after the selection (Steps 2 to 4). This is of particular importance with adherent cells, since cells killed by G-418 do not necessarily detach from the culture substrate.
   - After a subcultivation step, only viable cells will adhere to the culture substrate. Thus, subculturing facilitates the evaluation of the cultures and optimizes the culture conditions for the surviving cells.

3. If using trypsin to subculture the cells, you may perform the subcultivation without changing the culture dish, as follows:
   - After trypsin digestion, carefully remove the trypsin solution (but not the cells) with a fine-tipped Pasteur pipette.
   - Resuspend cells in 6 ml culture medium that contains G-418 plus either serum or a trypsin inhibitor.
   - Continue to incubate the cells in the same culture dish.

Cloning of Transfected Cells

After successful transfection and selection, you may clone (neo^r) transfectants. Single-cell cloning ensures that all (neo^r) transfectants are derived from the same parental cell. You may use any of several methods for single-cell cloning (*e.g.*, limiting dilution or picking individual cell colonies).

⚠ ***Even though you attempt to ensure that the cells are in single-cell suspension prior to plating, colonies may still arise from two cells that have stuck together. Therefore, cloning should be done at least twice ("recloning") to ensure a clonal population.***

The media formulations used in this protocol are the same as those for standard culture of the cell type or those used for the selection protocol (see section, **Selecting Transfected Cells***). You will need to decide whether to add the G-418 to the media, based on the requirements of your experiment.*

Cloning by Limiting Dilution (Suspension Cells or Adherent Cells)

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

⚠ ***Cloning involves preparing single-cell suspensions and plating cells at low densities. Therefore, adherent cells should be subcultured (****e.g.****, using trypsin or Dispase*) prior to this protocol.***

1. Carefully resuspend the cells in media.

2. Plate cells in 1:10 dilution steps starting from 10^5 cells/ml in a multiwell culture plate (tissue-culture grade, 96- or 24-well) to approximately 1 cell per well (lowest dilution).
   - We recommend 200 µl culture medium (for 96-well plates), or 1 ml culture medium (for 24-well plates).

3. Incubate cultures according to standard procedures until clones appear (usually within several days).
   - Use the dilution where a single stably transfected cell appeared.

4. According to the cell type, either subculture or passage the cells after an appropriate time (*e.g.*, when cells become confluent).

Cloning by Picking (Adherent Cells)

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

1. Plate adherent cells in 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
- approximately 1 to 1.5 × 10^4 cells (for a 60 mm Petri dish).

2. Incubate cultures according to standard procedures until colonies of 3 to 10 cells each appear (usually within several days).

3. Using an inverted microscope, place the tip of a fire-polished Pasteur pipette adjacent to the colony you want to pick.

4. Use suction to draw the cell colony into the tip of the pipette.

5. Transfer the picked colony into a fresh culture dish and culture as you would normally.

Modification for Picking Cells in Suspension

Use the above protocol for cells in suspension if you plate the cells in soft agar.

2.3. Parameters

Chemical Formula

$C_{20}H_{40}N_4O_{10} \times 2\ H_2SO_4$

Specific Rotation

104° to 121°, on dry basis in water (according to USP XXII).

Chemical Name

O-2-Amino-2,7-dideoxy-Δ-glycero-α-Δ-glucoheptopyranosyl[1->4]-O-3-deoxy-4C-methyl-3-[methyl-amino]-β-L-arabinopyranosyl-Δ-streptamine, disulfate salt

Chemical Structure

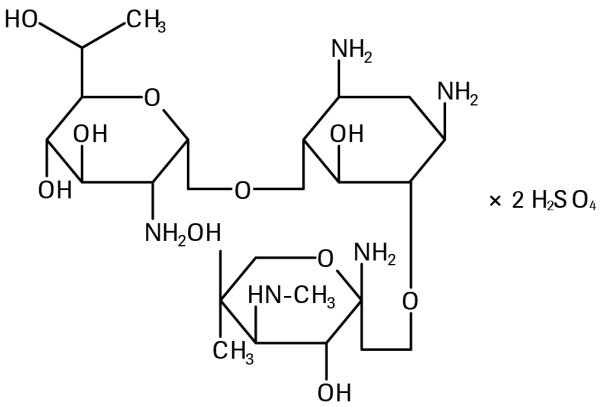

Fig. 1: Chemical structure of G-418.

Molecular Weight

692.72 Da

Working Concentration

Toxic Concentration

For each cell type, you must experimentally determine the concentration of G-418 to be added to the culture medium. To do this, perform the titration experiment (see section, **Determination of G-418 Concentration**) to generate a dose response curve and determine the amount of G-418 necessary to eliminate untransfected cells (toxic concentration). The titration should include G-418 concentrations between 50 µg/ml and 1 mg/ml.

⚠ ***Cells can escape selection if the concentration of antibiotic is too low or if the plating density is too high. It has been shown that the same cell line may show different sensitivity to G-418 depending on the culture media in which it is grown. Moreover, the sensitivity of non-resistant cells depends on the proliferative activity of the cells (i.e., rapidly proliferating cells are killed faster than slowly proliferating cells). Therefore, you should redetermine the optimal working concentration of G-418 whenever any of these experimental parameters are altered.***

Ideally, control cells should die within one week after the antibiotic is added, allowing colonies of resistant cells to form within 10 to 14 days. However, since sensitivity to G-418 varies with cell type, cell proliferative activity, culture media formulation, and other parameters, consider the protocol only as a guideline and modify it according to your test system. Be sure to take all the factors above into account when performing the titration experiment.

3. Results

Typical Analysis

Active weight: >700 µg/mg as determined with a microbiological assay using *Bacillus subtilis*.

4. Additional Information on this Product

4.1. Test Principle

How this Product Works

G-418, an aminoglycoside antibiotic, is used as a dominant selective agent in cell transfection experiments. The structure of G-418 resembles gentamicin, neomycin, and kanamycin. However, unlike these related compounds, G-418 interferes with the function of 80S ribosomes and blocks protein synthesis in eukaryotic cells. These aminoglycoside antibiotics can be inactivated by the bacterial aminoglycoside phosphotransferases APH(3')II and APH(3')I, which are encoded by genes on transposons Tn5 and Tn601 (903), respectively. Transfection of the neomycin-resistance gene(s) (neo) from either transposon Tn5 or Tn601 into cells will make the cells resistant to G-418 (neo^r) and enable the cells to grow in media containing G-418. This selection can be used on almost any cell type.

ℹ *Prototype vectors are pSV2neo and pRSVneo.*

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Cell Proliferation Reagent WST-1	8 ml, 800 tests	05 015 944 001
	25 ml, 2,500 tests	11 644 807 001
Cell Proliferation Kit I (MTT)	1 kit, 2,500 tests	11 465 007 001
Dispase® II (neutral protease, grade II)	5 x 1 g	04 942 078 001
Cell Proliferation Kit II (XTT)	1 kit, 2,500 tests	11 465 015 001
Dispase® I (neutral protease, grade I)	10 x approx. 2 mg, ≥ 20 U	04 942 086 001
DOTAP Liposomal Transfection Reagent	2 ml, 5 x 400 µl, 5 x 400 µg	11 202 375 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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