

More Convenience for Your Cell Culture Work: G-418 Solution and FuGENE® Transfection Reagents

Manfred Watzele* and Claudia Kirr

Roche Applied Science, Penzberg, Germany

*Corresponding author: manfred.watzele@roche.com

Introduction

Stable transfection of selectable markers

G-418, an aminoglycoside antibiotic, is used as a selective agent in cell transfection experiments. The structure of G-418 resembles that of 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 [1–3]. 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 will enable the cells to grow in media containing G-418. This selection can be used on almost any cell type.

The new G-418 solution is supplied sterile-filtered in a 50 mg/ml concentration and enhances the ease of use by saving time and minimizing handling steps. The use of the G-418 solution also increases safety, as it makes the weighing of hazardous G-418 powder unnecessary.

Materials and Methods

Thin layer chromatography (TLC) of G-418 samples

Fifty-microgram aliquots of G-418 samples from different suppliers were dissolved in distilled water at a concentration of 50 µg/ml and applied onto Silica gel 60 TLC plates

(Merck #105735.001) cut in 10 cm x 10 cm squares. The plates were developed in a solvent system containing EtOH:1-Butanol:NH₄OH (25%), in a 1:1:1 (v/v/v) mixture until the solvent front reached the top of the plate. After the plate had dried, the spots were visualized either by being stained in iodine vapor, or by being submerged in 0.3% ninhydrin solution and drying for 5 minutes at 110°C.

Determination of G-418 working concentration

PC-3 cells were grown in RPMI-1640 medium containing 2 mM glutamine and 10% fetal calf serum and plated at a density of 5 x 10⁴ cells/well in a 24-well plate. One day after plating, G-418 solution was added at different concentrations ranging from 50 µg/ml to 1000 µg/ml final concentration. Proliferation was measured 3 days after



Figure 1: Thin layer chromatogram of G-418 samples of different suppliers.

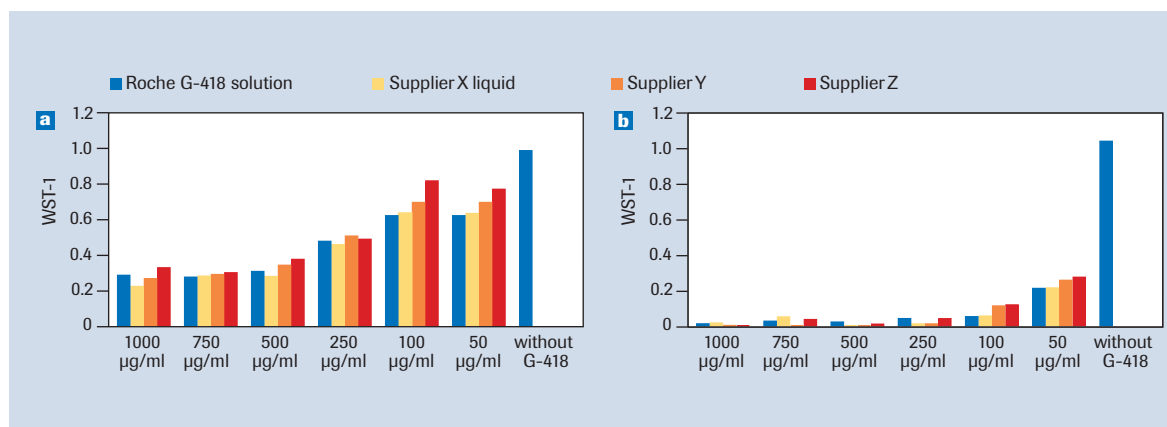
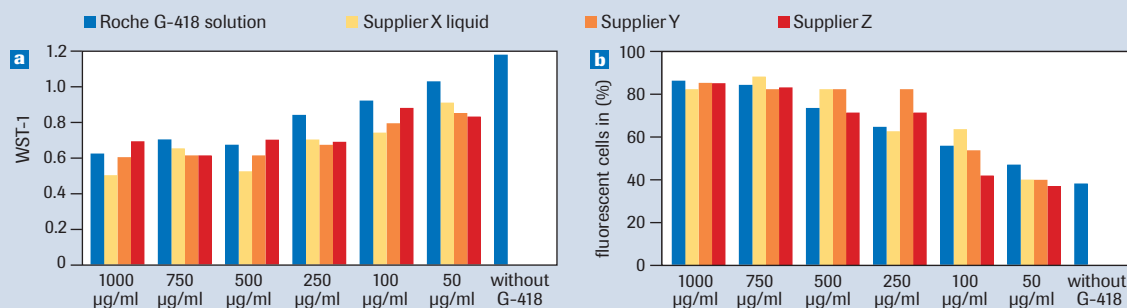


Figure 2: Treatment of PC-3 prostate cancer cells with different amounts of G-418. Cell proliferation was measured with WST-1 reagent after (a) 3 days and (b) 7 days.

Figure 3: Selection of transfected cells with different concentrations of G-418 (first round of selection).
(a) Proliferation and **(b)** percentage of fluorescent cells were determined.



G-418 exposure using the Cell Proliferation Reagent WST-1. Three days after the addition of G-418, cells were washed and trypsinized and one fourth of the cells were plated in fresh medium with the same G-418 concentrations as before. After 4 days, proliferation rates were measured again.

Transfection of PC-3 cells with FuGENE® 6

PC-3 cells were grown in RPMI-1640 medium containing 2 mM glutamine and 10% fetal calf serum and plated at a density of 5×10^4 cells/well in a 24-well plate 1 day prior to transfection. At the day of transfection, the cells were grown to 40% density and the transfection complex was added directly to the medium. The transfection complex was prepared as follows: 0.6 µl FuGENE® 6 and 0.3 µg of a plasmid carrying the eGFP gene and the neomycin resistance gene were added into 25 µl DMEM and incubated for 15 minutes at room temperature.

Selection of transfectants

One day after transfection, selection with G-418 solution was started using different concentrations of G-418 ranging from 50 µg/ml to 1000 µg/ml final concentration. Proliferation was measured 3 days after G-418 exposure using the Cell Proliferation Reagent WST-1. Parallel to this, fluorescent cells were counted. Three days after the

addition of G-418, cells were washed and trypsinized. One fourth of the cells were plated in fresh medium with the same G-418 concentrations as before. Four days after replating, fluorescent cells were counted again and proliferation was measured with the Cell Proliferation Reagent WST-1.

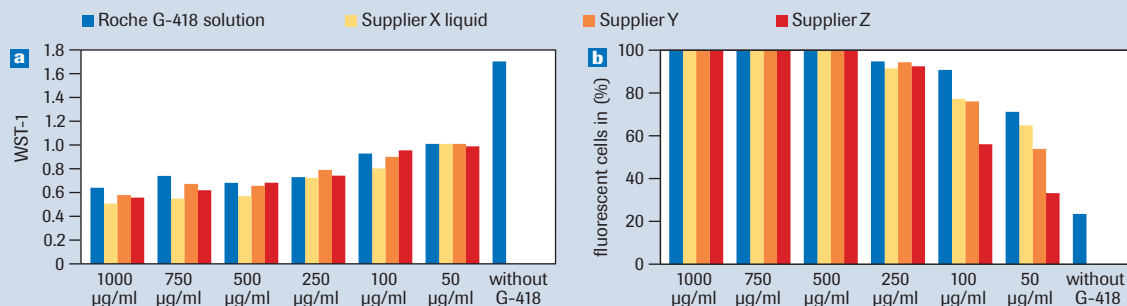
Results and Discussion

Purity of G-418

Important criteria for the specifications of G-418 as a selection agent for eukaryotic cells include high purity and a consistent, reliable activity that allows the use of established transfection and selection protocols. Since G-418 is often contaminated with other components, undesired cytotoxic side effects may arise from these contaminations.

We analyzed the new G-418 solution in comparison to products of other commercial suppliers. Since G-418 as well as potential contaminations are not colored or fluorescent, the different components are not easily detected. We therefore applied two different staining procedures. The thin layer chromatography shown in Figure 1 was stained with ninhydrin and detected considerable amounts of contaminations in one G-418-sample. The reagents of the other suppliers showed some minor contaminations, while the G-418 solution from Roche was nearly pure.

Figure 4: Selection of transfected cells with different concentrations of G-418 (second round of selection).
(a) Proliferation and **(b)** percentage of fluorescent cells were determined.



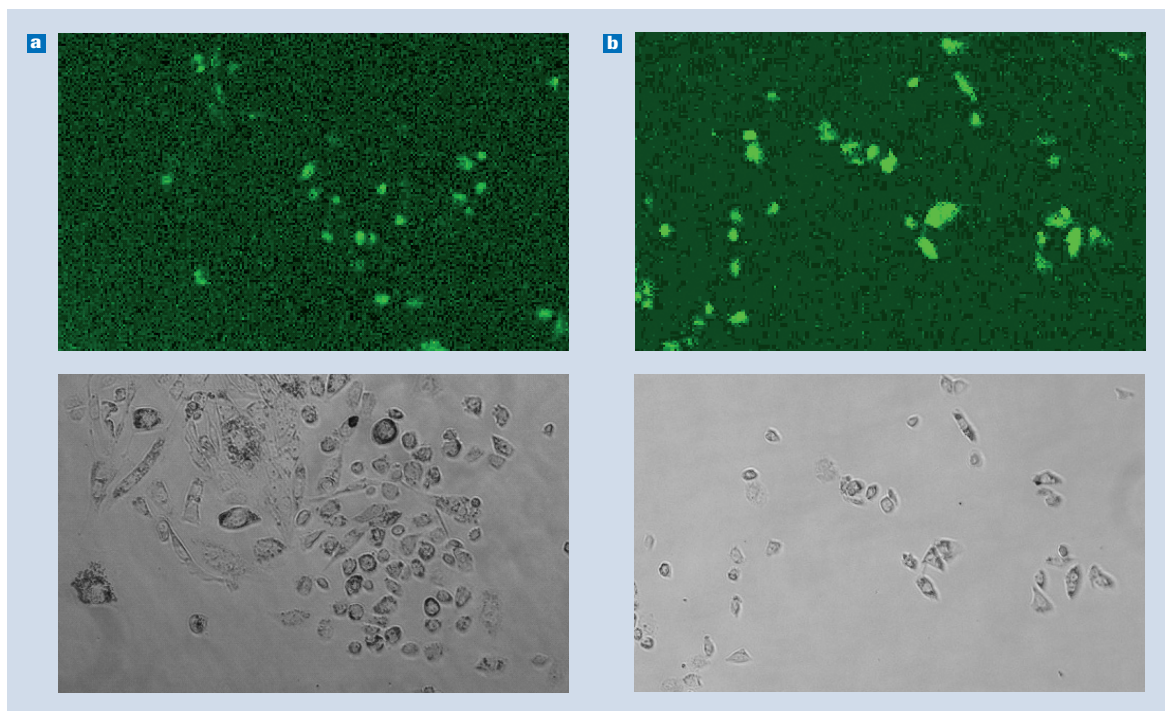


Figure 5: GFP fluorescence after selection of transfected cells (second round of selection).
(a) Unselected cells,
(b) G-418 selected cells.

Determination of the working concentration

In a dose-response curve different concentrations of G-418 solution were tested for their ability to stop growth of the adherent PC-3 prostate cancer cell line. The effect of G-418 is seen after 3 days (Figure 2a) and more pronounced after 7 days (Figure 2b). G-418 concentrations between 250 µg/ml and 500 µg/ml seem to be sufficient to completely block the growth of the non-transfected PC-3 cells.

Selection of transfected cells with G-418 solution

In order to determine the best conditions for a selection procedure after transfecting PC-3 cells with a plasmid bearing a neomycin resistance gene and the eGFP gene, G-418 concentrations between 50 µg/ml and 1000 µg/ml were applied. After a first round of selection, the proliferation rate of the untransfected cells is strongly diminished at G-418-concentrations >100 µg/ml (Figure 3a). At a concentration of >500 µg/ml G-418, >80% of the cells carry the eGFP-marker (Figure 3b), while in unselected cells the level was only 40 % (Figure 5a). After the second round of selection all cells that were cultured in the presence of >500 µl G-418 were fluorescent (Figure 5b), while the cells kept without G-418 or at a 50 µg/ml G-418 partially lost the ability to express eGFP (Figure 4b).

Conclusions

The new G-418 solution was potent even at low concentrations: 100 µg/ml G-418 were sufficient to select 90% of transfected cells. The new G-418 solution showed the highest proliferation rate among the reagents of all suppliers at all concentrations as determined in the WST-1

assay (Figures 3a and 4a). This high proliferation rate of cells transfected with a neomycin resistance gene is an additional proof that the new G-418 solution is not contaminated with cytotoxic compounds.

The product formulation as a sterile-filtered solution offers a high convenience for transfection experiments with minimal handling steps. In combination with FuGENE® 6, or the new FuGENE® HD transfection reagent, it allows a very fast selection of transfected cells within 1 week as shown for the PC-3 cell line. ■

References

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4. Anhalt JP, Brown SD (1978) *Clin Chem* 24: 1940–1947

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Product	Pack Size	Cat. No.
NEW!		
G-418 Solution	20 ml (1 g)	04 727 878 001
	5 x 20 ml (5 g)	04 727 894 001
FuGENE® 6	0.4 ml	11 815 091 001
Transfection Reagent	1 ml	11 814 443 001
FuGENE® HD	0.4 ml	04 709 691 001
Transfection Reagent	1 ml	04 709 705 001
	5 x 1 ml	04 709 713 001
Hygromycin B	20 ml (1 g)	10 843 555 001
Cell Proliferation Reagent WST-1	2,500 tests	11 644 807 001