Specification

Glycerol anhydrous *BioChemica* A1123

Synonym	1,2,3-Propanetriol
origin	from plants (non-animal origin!)
Density (d 20°C/4°C)	1.256 - 1.261
n 20°C/D	1.4740
Melting point	18°C
Formula	$C_3H_8O_3$
M	92.10 g/mol
CAS-No.:	56-81-5
HS-No.:	29054500
EC-No.:	200-289-5
Storage:	RT
LGK:	10 - 13
Disposal:	1
WGK:	1
Specification	
Assay (titr.)	min. 99.0 %
Organic chlorine	max. 0.0005 %
pH (5 M; H_2O)	5.5 - 8.0
Heavy metals (as Pb)	max. 0.0005 %
Insoluble matter	complies
Fatty acids	max. 0.02 %
Water (K.F.)	max. 0.5 %
Chloride	max. 0.0001 %
Sulfate	max. 0.001 %
A (1 cm/5 M in H_2O)	
260 nm	max. 0.05
280 nm	max. 0.05

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Literature

(1) Ogden, R.C. & Adams, D.A. (1987) *Methods Enzymol.* **152**, 61-87 Overview about the gel electrophoresis of DNA and RNA with recipes for buffers and solutions.
(2) Miller, H. (1987) *Methods Enzymol.* **152**, 145-170 Preparation of phage- and plasmid-DNA for storage as pure culture.
(3) Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning:* A Laboratory Manual. 2nd Edition. Page 16.32-35 (Glycerol shock); Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.
(4) Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl. K. (eds.) (1995) *Current Protocols in Molecular Biology.* Page 9.1.7 (Glycerol shock); Suppl. 36. Greene Publishing & Wiley-Interscience, New York.
(5) Wilson, S.P. & Smith, L.A. (1997) *Anal. Biochem.* **246**, 148-150 Addition of glycerol during DNA incubation increases the transfection efficiency.

Comment

Glycerol is a component of several buffers, media or reagents in biological research. It is supplied as glycerol anhydrous or 87 %, the latter containing 13 % water. Both glycerols are from the same quality, the 87 % glycerol has the advantage of a lower viscosity. Anhydrous glycerol can be pipetted with difficulty only, especially at low temperatures. Glycerol is sterilized by autoclaving (20 minutes, liquid cycle). At concentrations above 5 % it interferes with protein assays and above 40 % at 280 nm, respectively.
After the calcium phosphate transfection of mammalian cells, a glycerol or DMSO shock can be included to improve the transfection efficiency (3, 4). Both substances are toxic for the cells and the time of incubation has to be determined for each cell type. In a further development of the calcium phosphate method it is recommended to add glycerol during the DNA incubation (5). Another application is the purification of bacteriophage l with glycerol as separation medium (ref. 3 page 2.78).
Freezing and storage of eukaryotic cells: Glycerol may be used instead of DMSO for the freezing of eukaryotic cells. It prevents the formation of ice crystalls and prevents damage of the cells. Glycerol is used at a concentration of 10 %. It is highly toxic for the cells! Therefore, the 'freezing medium' should be cooled on ice. The freezing medium is either the 'normal' culture medium with the addition of 10% glycerol or 90 % serum plus 10 % glycerol, which increases the viability. Most of the cell types are not directly transferred to the liquid nitrogen. They have to be cooled down slowly (refrigerator, -70°C freezer well packed, liquid nitrogen). Thawing of cells has to be done as quick as possible. It is recommended to wash the cells once with medium before plating or change the medium two days after thawing.
Freezing and storage of bacteria (2): Depending on the technique, glycerol is used at a concentration ranging from 15 - 50 %. Method I: 0.15 volume of sterile glycerol is added to 0.85 volume of liquid culture (e. g. in LB medium). The mixture is frozen in an ethanol/dry ice bath or liquid nitrogen. Store the frozen stock at -70°C. Method II: One colony is removed from an agar plate or the pelett of an over night culture is thoroughly mixed with TM buffer (10 mM Tris · HCl (pH 7.4), 10 mM $MgSO_4$, 50 % glycerol) and frozen as described above.
For recultivation of bacteria do not thaw the complete vial. It is sufficient to scratch with a steril tooth pick over the surface of the frozen stock to inoculate culture medium.