Name:	**GVB^{++} (with Ca^{++} and Mg^{++})**
Catalog Numbers:	**B100 and B102**
Sizes Available:	**1000 mL and 250 mL**
Composition:	0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN_3 , pH 7.3. Containing 0.15 mM calcium chloride and 0.5 mM magnesium chloride
Form:	Liquid
Buffer:	Sodium veronal
Preservative:	Sterile filtered with 0.025% sodium azide as a bactericidal agent
Storage:	+4°C Avoid freezing which causes gelatin to gel. If frozen, heat to redissolve gelatin.
Precautions:	Azide is poisonous to all living organisms.
Origin:	Manufactured in the USA.

General Description

GVB^{++} is the traditional buffer used in complement assays. Clinical CH50 assays are performed in this buffer and almost every laboratory that performs complement assays performs them in this buffer. It is supplied as an assay-ready buffer with all the components present at the proper concentrations for CH50 assays. The concentrations of metal ions are the traditional concentrations and these give maximal complement activation. Dilutions of serum and other assay components for CH50 assays are prepared in this buffer (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

Ordering Requirements

Buffers such as GVB^{++}, GVB^{o}, and GVBE need to be ordered by Friday in order to receive them the next week. They are shipped Monday afternoon by overnight courier for delivery on Tuesday or Wednesday. They can usually be used for 3 months after preparation if kept cold @ 4°C. They are shipped cold, but are not harmed at room temperature and must be warmed to 37°C for assays.

Buffer Components

Veronal is used as the buffer because in the mid-1900s this was the only buffer for pH range 7.2-7.4 that did not chelate metal ions and did not to inhibit complement reactions as did other buffers. Sodium chloride is present to provide an isotonic environment so that cells do not lyse due to osmotic pressure. Gelatin is present to prevent loss of protein components due to adsorption onto tips or tubes during dilutions and in the assays themselves. Azide is present to prevent bacterial growth. Calcium is present because the classical and lectin pathways require it to hold the subunits of the C1, MBL and ficolin complexes together. Magnesium is required for formation of the C3 and C5 convertases of all three pathways of complement.

Although total calcium in plasma and serum is about 2.3 mM, the free available concentration is about 1.07 mM. The remainder is complexed to proteins or small molecules. Complement buffers contain 0.15 mM calcium because it was found that under CH50 assay conditions the classical pathway activity is greater at 0.15 mM than at 1.0 mM calcium. Plasma and serum concentrations of total magnesium are about 0.87

mM and the free available concentration is 0.5 mM. Thus, GVB^{++} contains 0.5 mM magnesium chloride.

Physical Characteristics

The concentration of gelatin in this buffer is below the concentration that forms solid gels. However, because of the gelatin is close to its gelling concentration at 4°C some strings of gelatin form during standing at this temperature. They can be redissolved easily by heating to 37°C or by brief heating in a microwave oven.

Applications

GVB^{++} should be used to prepare EA and to wash them before their use in assays. GVB^{++} is used to dilute all of the components of classical and lectin pathway assays.

References

Morgan, B.P. ed. (2000) Complement Methods and Protocols. Humana Press.

Dodds, A.W. and Sim, R.B. editors (1997). Complement A Practical Approach (ISBN 019963539) Oxford University Press, Oxford.

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