Name:	**VBS^{++} (with Ca^{++} and Mg^{++})**
Catalog Numbers:	**B112 and B113**
Sizes Available:	**250 mL and 1000 mL**
Composition:	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, plus 0.025% sodium azide as a bactericidal agent
Storage:	$+4^oC$ If frozen, warm and mix to redissolved salts and buffer.
Precautions:	Azide is poisonous to all living organisms.
Origin:	Manufactured in the USA.

General Description

VBS^{++} is like the traditional buffer used in complement assays (GVB^{++}) but it lacks the carrier protein gelatin often used in these assays. GVB may be replaced with VBS if gelatin is undesirable or may interfere with the assays. The concentrations of metal ions are the traditional concentrations and these give maximal complement activation (see Buffer Components section below). Dilutions of serum and other assay components for CH50 assays may prepared in this VBS^{++} buffer (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

Ordering Requirements

Buffers such as VBS^{++}, $SGVB^{++}$, 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^oC. They are shipped cold, but are not harmed at room temperature and must be warmed to 37^oC 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 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. 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 buffer is stable at 4°C and should be usable for 3 months from the time of manufacture. The salts in this buffer may crystalize if the buffer is frozen. They can be redissolved easily by heating to 37°C or by brief warming in a microwave oven. The solution should be thoroughly mixed after these procedures. Extended time above 4°C may shorten the time this buffer will resist bacterial growth.

Applications

VBS^{++} may be used when a buffer free of carrier proteins is needed for diluting proteins or as the buffer for complement assays. VBS^{++} may also be used to prepare GVB^{o}, GVB^{++}, and GVBE or it may be used to prepare similar complement buffers that include different carrier proteins in place of gelatin in situations where gelatin interferes with assays. BSA (0.1%) or another carrier protein may be used for this.

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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