Name:	**$SGVB^{++}$ (with Ca^{++} and Mg^{++})**
Catalog Numbers:	**B110 and B111**
Sizes Available:	**250 mL and 1000 mL**
Composition:	170 mM Sucrose, 0.1 % gelatin, 5 mM Veronal, 57 mM NaCl, 0.025 % NaN_3 , 0.15 mM calcium chloride, and 0.5 mM magnesium chloride, pH 7.3.
Form:	Liquid
Buffer:	Sodium veronal
Preservative:	Sterile filtered, plus 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

$SGVB^{++}$ is a specialized buffer which is low in ionic strength (due to the low NaCl concentration), but isotonic (due to the sucrose). This combination of properties allows complement assays or binding assays to be performed with cells at low ionic strength, which improves binding, while the sucrose keeps the cells from lysing due to the high concentration of sucrose. This buffer is practically identical to DGVB buffer except that they use D-glucose instead of sucrose to maintain their isotonic characteristic. These buffers are isotonic with VBS, GVB, PBS, etc. meaning that the osmotic pressure on cell membranes will be the same even though the salt concentration is low. $SGVB^{++}$ buffer is especially useful in C1 assays (Dodds, A.W. and Sim, R.B. (1997)) and in binding assays such as those measuring the binding of factor B or factor H to EsC3b cells (sheep erythrocytes bearing surface-bound C3b) (Pangburn, M.K. and Muller-Eberhard, H.J. (1978); Pangburn, M.K., et al. (1980)). These interactions are sensitive to the salt concentration and lowering the ionic strength can enhance binding 5- to 10-fold.

Ordering Requirements

Buffers such as $SGVB^{++}$, VBS^{++}, 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 are usually 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 and sucrose are 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 with 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, $SGVB^{++}$ contains 0.5 mM magnesium chloride.

Physical Characteristics

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

Applications

$SGVB^{++}$ can be used to titer both purified C1 complex (CompTech #A098) and C1 in normal human serum samples (Dodds, A.W. and Sim, R.B. (1997)). In these assays all components are diluted in $SGVB^{++}$ and the EA cells are washed into this buffer. Binding assays such as those measuring the binding of C5, factor B or factor H to EsC3b cells (sheep erythrocytes bearing surface-bound C3b) or ZymosanC3b cells also benefit from being performed in $SGVB^{++}$ (Pangburn, M.K. and Muller-Eberhard, H.J. (1978); Pangburn, M.K., et al. (1980)). The enhancement of binding can be as much as 10-fold compared to using a buffer with physiological ionic strength such as GVB or PBS.

References

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

Pangburn, M.K. and Muller-Eberhard, H.J. (1978) Complement C3 convertase: Cell surface restriction of fi1H control and generation of restriction on neuraminidase-treated cells. Proc. Natl. Acad. Sci. USA 75:2416-2420.

Pangburn, M.K., David C. Morrison, D.C., Robert D. Schreiber, R.D. and Hans J. Muller-Eberhard, H.J. (1980) Activation of the Alternative Complement Pathway: Recognition of Surface Structures on Activators by Bound C3b. J. Immunol. 124:977-982.

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