Name:	**Es (sheep erythrocytes)**
Catalog Numbers:	**B210**
Sizes Available:	**20 ml**
Concentration:	5×10^8 cells/mL
Absorbance:	$A_{412\ nm} = 0.87$ at 1/50 dilution in deionized water
Form:	Liquid – DO NOT FREEZE
Activity:	Do not activate classical or alternative pathways of complement
Buffer:	GVB^o (CompTech #B101 and B103)
Preservative:	GVB^o contains 0.025% sodium azide as a bactericidal agent
Storage:	$+4^oC$ Avoid freezing. Freezing lyses the cells.
Source:	Normal sheep blood from USDA registered facility.
Origin:	Manufactured in the USA.

General Description

Sheep red blood cells which have been washed free of sheep complement proteins, but were not coated with hemolysin antibodies. These cells have traditionally been used as negative controls for assays of the human classical and alternative complement pathways in serum samples (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). Most human serum samples have small amounts of natural antibodies (usually IgG) to sheep antigens and at high concentrations of serum will agglutinate Es and activate the classical pathway leading to lysis. This rarely occurs at dilutions used in the CH50 titration assay where serum is diluted more than 1/100.

Es are supplied at assay-ready concentrations in the traditional buffer used in CH50 assays (GVB^{++}). They are prepared fresh every Monday morning and need to be ordered the previous Friday in order to receive them the next week. They are shipped Monday afternoon by overnight courier for delivery on Tuesday (or Wednesday for most international shipments). They can usually be used for 2 weeks after preparation. They are shipped cold, but are not harmed by extended periods at room temperature (note that they circulate 60-90 days a 37^oC *in vivo*). They should be washed once before each use (5 min at 500 to 1000 x g at 4^oC) and resuspended in GVB^{++} to reduce background. This procedure may also be used to concentrate the cells.

Physical Characteristics

Es are natural uncoated erythrocytes that do not activate complement under typical assays conditions. They do not have IgG or IgM on their surface, however, there are natural antibodies in most animal serum. In the most sensitive assays it has been estimated that as few as 10 IgM/EA can cause lysis while this requires approximately 1000 IgG/EA (Ross, G.D. (1986)). This is primarily because in IgM there are five closely spaced Fc domains in fixed positions on each molecule. The spacing of IgG molecules is more random and it is relatively rare to find two or more IgG in the correct positions on the surface of a cell.

Applications

Es cells are primarily used as controls for CH50 assays and for specialized tests requiring uncoated erythrocytes. Natural antibodies present in human blood to animal antigens may cause agglutination of the cells and lysis if the serum is used at high

concentrations, however, this does not interfere with the CH50 titers because EA used in these assays already carry high levels of IgM antibodies and serum is diluted 100-fold or more. IgG antibodies are 100-fold less active than IgM and most of the cross-reactive antibodies in human blood are IgG.

Regulation

Sheep erythrocytes (Es) are used for human complement assays partly for convenience, but also because they lack membrane-bound regulators of human complement. No significant level of functional DAF, CD59 or CR1 exists on Es for human complement. Thus, Es are useful for their lack of membrane regulatory activities.

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.

Ross, G.D. (1986) Immunobiology of the Complement System. (ISBN 0-12-5976402) Academic Press, Orlando.

FOR RESEARCH USE ONLY.
NOT FOR HUMAN OR DRUG USE.

Complement Technology, Inc.
4801 Troup Hwy, Suite 701
Tyler, Texas 75703 USA
Phone: 903-581-8284
FAX: 903-581-0491
Email: contactCTI@aol.com
Web: www.ComplementTech.com