Name:	**EA (antibody-sensitized sheep erythrocytes)**
Catalog Numbers:	**B200, B201 and B202**
Sizes Available:	**5 mL, 10 mL and 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:	Fully functional in complement CH50 assays
Buffer:	GVB^{++} (CompTech #B100 and B102)
Preservative:	GVB^{++} 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 coated with rabbit anti-sheep erythrocyte antiserum (hemolysin) have traditionally been used to assay the activity of the complement classical pathway in serum samples. These assays yield the CH50 titer of the serum (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

EA 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 by 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 (3 min at 500 to 1000 x g at 4^oC) and resuspended at the original concentration in GVB^{++} to reduce background. This procedure may also be used to concentrate the cells. This may be accomplished by reducing the volume used to resuspend the cells.

Physical Characteristics

EA are made with IgM antibodies for the following reason. IgM is approximately 100 times more efficient at activating the classical pathway than IgG because C1q must bind to at least two Fc domains on antibodies for activation to occur. Thus, in the most sensitive assays it has been estimated that as few as 10 IgM per EA can cause lysis while with IgG lysis requires approximately 1000 IgG per EA (Ross, G.D. (1986)). This is primarily because in IgM there are five closely spaced Fc domains in fixed positions on each molecule. Because the spacing of IgG molecules on a surface is more random, it is less probable to find two to five IgG molecules in the correct positions on the surface of a cell to bind and activate C1.

Assays

Although the CH50 titer is widely used and serves as the primary clinical test for complement activity, the CH50 assay procedure is not entirely standardized. In research labs there are as many procedures as there are labs, but all are basically similar and give useful results. A detailed step-by-step typical CH50 titer method may be obtained from CompTech upon request. Briefly, a complement-containing serum sample is diluted so

that the final dilutions in the assay are in the range from 1/100 to 1/500. Controls include two tubes with no complement for the 0% lysis background and two tubes in which water is used in place of buffer for the 100% lysis control. Assay tubes should be set up while on wet ice. The volume is brought to 1.3 mL with GVB^{++}(or water), the tubes are mixed and 0.2 mL of cells at 5 x 10^8 EA/mL are added and mixed. The reactions should be incubated for 60 min in a 37^{o}C water bath with remixing at approximate 10 min intervals. After one hour, the remaining cells are spun down (500-1000 x g for 3 min) and the absorbance of the supernatant determined at 541 nm in a 1 cm cuvette. There are a variety of mathematical methods for graphing the results. An almost perfect straight line should be obtained by plotting on a log-log plot the microliters of serum used in each assay on the x axis and y/(1-y) on the y axis. The term "y" refers to the proportion of cells lysed and would be zero at 0 % lysis, 0.3 at 30 % lysis ,0.5 at 50% lysis and so on. The CH50 value of the complement sample is obtained from the point where the line crosses 1.0 on the y axis (50 % lysis) and is calculated as follows: CH50 = 1000 divided by the volume (in µL) of serum giving 50% lysis. For example, if 50% lysis was achieved with 5.6 µL of serum then the CH50 = 179 units/mL. It should be noted that different CH50 values can be obtained on what should be identical samples. This is the result of the vast number of variables involved in CH50 determinations including the fact that sheep erythrocytes harvested in the spring are more easily lysed than cells from blood drawn in the fall, no two hemolysin antibody lots are the same because they are made from antiserum raised in rabbits, etc.

Applications

EA may be used to determine the CH50 hemolytic titer of serum, that is, the CH50 (see Assays above). They can be used to titer the functional activity of individual complement components using depleted serums. They may also be used to prepare cells carrying activated complement intermediates (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). EA are incubated with C1 to make (EAC1) or C1 and C4 (EAC14). EAC14 cells may be incubated with C2 to prepare EAC142 cells bearing the classical pathway C3 convertase and EAC142 may be incubated with C3 to prepare EAC1423 cells which carry the C5 convertase. Each intermediate may be used to assay fluid phase components for functional complement activity. For example, lysis of EAC142 cells is extremely sensitive to low C3 concentrations and this can be used to titer C3 complement activity even in complex mixtures such as serum.

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.

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