Name:	**Er (rabbit erythrocytes)**
Catalog Numbers:	**B300, B301 and B302**
Sizes Available:	**5 mL, 10 mL and 20 mL**
Concentration:	5×10^8 cells/mL
Absorbance:	$A_{412\ nm} = 1.10$ at 1/50 dilution in deionized water
Form:	Liquid – DO NOT FREEZE
Activity:	Er activate the 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 rabbit blood from USDA registered facility.
Origin:	Manufactured in the USA.

General Description

Er are rabbit red blood cells which have been washed free of rabbit plasma proteins, but they are not coated with antibodies as is done with EA. These cells have traditionally been used as cells that spontaneously activate the human alternative complement pathway 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 rabbit antigens and at high concentrations of serum these will agglutinate Er. This will activate the classical pathway if not blocked. Blocking the classical pathway is usually accomplished with 3 to 13 mM MgEGTA (CompTech 0.1 M MgEGTA #B106).

Er are supplied at assay-ready concentrations in the traditional buffer used for APH50 assays (GVB^o) which lacks metal ions. 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 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+ days at 37^oC *in vivo*). They should be washed once before each use (3-5 min at 500 to 1000 x g at 4^oC) and resuspended in GVB^o to reduce background and readjust their concentration. This procedure may also be used to concentrate the cells.

Physical Characteristics

Er are natural uncoated (non-opsonized) rabbit erythrocytes.

Assays

Although the APH50 titer is widely used and serves as the primary test for alternative pathway complement activity, the APH50 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 APH50 titer method may be obtained from CompTech upon request.

The main difference between the classical pathway assays for CH50 determination and the alternative pathway assays for APH50 is the concentration of serum required. Alternative pathway activity becomes ineffective at dilutions of serum

beyond 1/10 to 1/30. In contrast, CH50 titers are performed in the range 1/100 to 1/500 dilution. Dilution silences the alternative pathway in CH50 titers, but APH50 measurements would be overwhelmed by the classical pathway if this pathway were not blocked. Blocking of alternative pathway activity may be accomplished by using serum depleted of factor B or D or most conveniently by adding 3 to 13 mM MgEGTA (final concentration) to the assays which completely inhibits the classical pathway by chelating calcium ions and dissociating C1.

Briefly, a complement-containing serum sample is diluted such that the final dilutions in the assay are in the range from 1/2 to 1/20. Controls include two tubes with no complement for the 0% lysis background and two tubes for 100% lysis. Assay tubes should be set up on ice. The assays are performed in 200 µL containing 4 to 60 µL of test serum, 10 µL 0.1 M MgEGTA, GVB^{o} , and 50 µL Er (CompTech #B300). The assays are mixed and placed in a 37ºC water bath with remixing at approximately 10 min intervals. After 30 min, 2 mL of cold GVBE is added, mixed and the unlysed cells are spun down (500-1000 x g for 3 min). The absorbance of the supernatant should be determined at 412 nm in a 1 cm cuvette and the percentage of specific lysis calculated after subtracting the background and dividing by the 100% lysis control. The data is plotted as percent lysis on the y axis and µL serum on the x axis. The APH50 value of the complement sample is calculated from the amount of serum needed to cause lysis of 50% of the cells. For example, if it takes 12 µL NHS to lyse 50% of the cells then this serum would contain 83 AHP50 units/mL (1000 µL /12 µL = 83 units/mL). It should be noted that somewhat different APH50 values can be obtained on what should be identical samples. This is the result of the vast number of variables involved in APH50 determinations including the fact that each batch of rabbit erythrocytes is slightly different.

Applications

Er cells are primarily used to determine the activity of the alternative pathway of complement (APH50 titer) (Morgan, B.P. (2000; Dodds, A.W. and Sim, R.B. (1997)). Natural antibodies present in human blood to animal antigens may cause agglutination of the cells. This antibody may also cause lysis if the classical pathway is not blocked.

Rabbit erythrocytes are attacked and lysed by the alternative pathways of most mammalian species. It has not been clearly demonstrated why rabbit complement does not lyse rabbit Er when most other animal species do lyse these cells, although they do carry rabbit DAF which works on rabbit, but not human complement enzymes.

Regulation

Rabbit erythrocytes (Er) 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 Er for human complement. Thus, Er 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.

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