ARTHRITIS FOUNDATION – CENTERS FOR DISEASE CONTROL REFERENCE REAGENT
FOR IgM ANTI-CARDIOLIPIN ASSAY AGAINST EPITOPE ON BETA-2 GPI

Product Package Insert  Catalogue IS2718  Lot 05-0059  9/20/05

Human IgM Anti-Cardiolipin Monoclonal Antibody
EY2C9 20 µg/ml

Intended Use

For in vitro immunodiagnostic use as an external control in solid phase enzyme immunoassays detecting beta 2-glycoprotein 1 (β2 GPI) dependent IgM anti-cardiolipin (aCL) antibodies.

Description of Reference Reagent

EY2C9 is a monoclonal IgM aCL antibody established from peripheral blood lymphocytes of patients with anti-phospholipid syndrome (APS), using a combined technique of EBV transformation and somatic cell hybridization. These monoclonal human IgM aCLs have specificities similar to those of aCLs in sera from patients with APS.1 EY2C9 with a protein concentration of 41.4 µg/ml was obtained from Drs. K. Ichikawa and T. Koike at the Hokkaido Univ. School of Medicine in Sappora, Japan. It was diluted to a protein concentration of 20.0 µg/ml with 10mM Tris buffered saline, pH 7.4 with 1% BSA. Volumes of 0.50 ml were dispensed in borosilicate vaccine vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. The vial contents were sterile as determined by U.S.P. approved methods of sterility testing. Vials are stored at the CDC at –20°C. The mean dry weight and SD of material in 19 randomly selected vials after freeze-drying was 7.14 ± 0.16 mg.

Reconstitution and Storage

Store the freeze-dried material at –20°C until use. To reconstitute, the contents should first be shaken to the bottom by tapping of the upper end. Before the stopper is removed, the vacuum should be broken by insertion of a hypodermic needle through the rubber stopper. Precisely 0.50 ml of distilled water should then be added, and the vial restoppered. The freeze-dried powder should dissolve readily with gentle swirling (avoid form). Allow to stand at least 30 minutes before use and store at 4°C until use, not later than 24 h after reconstitution. Note: Contents of vials that have been stored at high temperatures may not completely go into solution. These vials should be discarded. Although not recommended, the reconstituted material will withstand at least 4 weekly freeze-thaws without loss of activity. If future use of reconstituted material is contemplated, portions of the undiluted material sufficient for a single use should be store at –70°C and discarded after use.

Antibody Content

In the presence of β2GPI, EY2C9 bound to the wells of cardiolipin-coated microtiter plates in a dose-dependent manner and reacted with β2GPI on oxygenated polystyrene plates. Seven international reference laboratories using a variety of IgM anti-cardiolipin ELISA procedures, both in-house and commercial kits, tested EY2C9 at the dilution used when routinely testing their samples. The variability of anti-cardiolipin is well known, therefore it is recommended that results be reported as protein concentration of µg/ml or ng/ml. However, when expressed in MPL units, a median value of 44 MPL units with a modified range of 16 – 145 MPL units was reported with OD values ranging from 0.577 to 2.387. Two laboratories ran EY2C9 in their IgM anti-β2-GPI assays. They reported concentrations of 151 and 186 arbitrary units (AU) with OD values of 1.820 and 2.352.
Suggested procedure for Standardization of Quality Control Reagents

Monoclonal antibodies can provide an external control of the technical variations both between various kits as well as in day to day assays. Since the amount of the reference preparation is limited, it should be used to calibrate secondary standards that can be run each day along with other samples being analyzed. The monoclonal antibody should be serially diluted to produce a standard curve. Based on these results, it is recommended that 2 different concentrations, one with results expected in the cut off range and the other in the medium positivity range be selected for use as controls.

To calibrate a secondary standard (i.e., a positive control to be included in subsequent routine test runs):

1. Reconstitute ampule as described above.
2. Prepare doubling diution of the reference reagent and the secondary standard from 1:10 to 1:1280.
3. Carry out the routine test procedure on the dilutions.
4. Determine the points in the cutoff and medium positivity range.
5. Relative potency = \( \frac{\text{Reciprocal of endpoint dilution of secondary standard}}{\text{Reciprocal of endpoint dilution of monoclonal antibody}} \)

If the secondary standard is stable, the relative potency obtained should not change. Any changes in day to day performance of the secondary standard are more likely due to changes in test performance. Generally, in the day to day performance of the test, several dilutions of the secondary standard are run. A single dilution, unless it is run at or near its end point, may not reveal major changes test performances.

These reference reagents can also be used to determine the most suitable or sensitive method, kit or reagents; to compare lot to lot changes; as a common reference for interlaboratory comparability; and for comparison of results with that of the laboratories which initially evaluated the reference reagent.

Reference:

ANA Reference Materials Laboratory
c/o Robert Vogt, Mailstop F-19
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30341
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WWW.AutoAb.org