CERTIFICATE OF ANALYSIS

Complement Technology, Inc.
4801 Troup Hwy, Suite 701
Tyler, Texas 75703, USA

Product: MCAR
Catalog # **B250** **Lot #**
Exp. Date:

Description: MCAR (Mouse Complement Assay Reagent)

Specifications	**Limits**	**Results**
PROTEIN CONCENTRATION	> 0.40 mg/mL Using an extinction coefficient of $E^{1\%}/_{280}$nm = 14.0	0.53 mg/mL
FILL VOLUME	1.0 – 1.1 mL	1.07 mL
BUFFER	Veronal Buffered Saline (VBS) pH 7.3 ± 0.1	Conforms
FUNCTIONAL ACTIVITY	See attached sheets	Must be titrated with each mouse serum sample used.
PRESERVATIVE	0.025 % sodium azide	Conforms
FILTRATION	0.45 µm filter	Conforms
SPECIFICITY	Sensitizes sheep erythrocytes for activation of mouse complement classical pathway	Conforms

Short term storage (≤7 days) at 4°C.
Longer term storage at ≤ -80°C.
Thaw quickly at 37°C, mix, and put in an ice+water bath to cool.
Avoid Repeated Freeze/Thaw

FOR RESEARCH USE ONLY
NOT FOR HUMAN OR DRUG USE

Signature of Analyst

Date of Analysis

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Complement Assay Procedure for Mouse Classical Pathway

From: Michael Pangburn, Complement Technology, Inc., 7/2012 (www.ComplementTech.com)

Mouse complement is famously difficult to assay. Mouse serum rapidly loses its activity and the typical titer of mouse serum is far below the 150-200 CH50 units/mL found with human serum. Mouse strains differ greatly in their complement CH50 titers (Ref 1, 2 & 3) and a great many common mouse strains are genetically deficient in C5 (Ref 4 & 5) meaning that they cannot lyse cells even if they have an otherwise fully functional complement system. The exact reasons for low titers in C5 sufficient strains are not entirely clear and they may be different for different mouse strains. However, a common reason is that mouse classical pathway components are not efficiently activated by the standard antibodies bound to EA. At Complement Technology, Inc. we have developed a unique hemolytic reagent (MCAR = Mouse Complement Assay Reagent) that sensitizes sheep erythrocytes to provide improved mouse CH50 titers. For example, one commercially available sample of mouse complement exhibited a greater than 10-fold increase in hemolytic titer (CH50) with MCAR present compared to assays without MCAR. At this time we cannot say how many mouse strains this reagent will work with, but we have used MCAR with three and it worked equally well with all.

Mouse serum is extremely unstable. We have observed a rapid loss of activity after thawing (100% loss if left at 4^{o}C overnight). Thus, we advise rapidly thawing of NMS, aliquoting it and re-freezing it immediately. Upon use, thaw only when the experimental setup is ready for the NMS sample and keep it on wet ice.

Every lot of mouse complement will be different. Preliminary assays will need to be done to determine the correct amount of NMS and MCAR to use. Normal sensitized sheep cells (EA) work well and the lysis is boosted by MCAR. Most NMS samples generally contain a lot of hemoglobin and the more serum used the higher the background. A sample assay protocol is shown in the table below where the total assay volume was kept to a minimum at 100 uL/assay. In general, tubes are set up with buffers and MCAR at room temperature. Then EA are added, mixed and incubated 5 min at room temperature. After MCAR has incubated with the EA cells for 5 min or more at room temp the NMS is added to start the assay. The tubes should be mixed and moved to a 37^{o}C water bath for 30 min with occasional (every 5-10 min) resuspension by vortexing.

One word of caution, do not centrifuge the EA after they have been mixed with MCAR and do not make a master mix of EA and MCAR and then pipette into the tubes. These procedures will result in inconsistent results.

After 30 min at 37^{o}C, the assays are diluted with cold GVBE, mixed by vortexing, centrifuged to pellet the unlysed cells and 200 uL of the supernatant is transferred to a flat-bottomed microplate and the absorbance read at 415 nm. Alternatively a microcuvette and spectrophotometer may be used.

EA are used at 5 x 10^{8}/ml in GVB++ and all dilutions are done in GVB++ buffer.

References

1. Ong G.L. and Mattes M.J. Mouse strains with typical mammalian levels of complement activity. J Immunol Methods 125:147-158, 1989.
2. van Dijk, H. et al. Estimation of classical pathway of mouse complement activity by use of sensitized rabbit erythrocytes. J Immunol Methods 39:257-268, 1980.
3. Terry W.D. et al. Differences in serum complement activity among inbred strains of mice. J Immunol. 92:576-578, 1964.
4. Nilsson U.R. and Muller-Eberhard, H.J. Deficiency of the fifth component in mice with an inherited complement defect. J Exp. Med. 124:1-16, 1967.
5. Wetsel, R.A. et al. Deficiency of the murine fifth complement component (C5). A 2-base pair gene deletion in a 5'-exon. J. Biol. Chem. 265:2435-2440, 1990.

Protocol for Titering Normal Mouse Serum and MCAR												
											Mix &	
											Spin	
						5 min	Mouse		30 min	Cold	&	%
#	GVB++	H2O	GVBE	MCAR	EA	21oC	Serum	Mix	@ 37	GVBE	A415	Lysis
1			70 uL	10 uL	10 uL		10 uL			200 uL		
2			70 uL	10 uL	10 uL		10 uL			200 uL		
3	90 uL				10 uL					200 uL		
4	90 uL				10 uL					200 uL		
5		80 uL			10 uL		10 uL			200 uL		
6		80 uL			10 uL		10 uL			200 uL		
7	85 uL			0 uL	10 uL		5 uL			200 uL		
8	80 uL			0 uL	10 uL		10 uL			200 uL		
9	70 uL			0 uL	10 uL		20 uL			200 uL		
10	60 uL			0 uL	10 uL		30 uL			200 uL		
11	80 uL			5 uL	10 uL		5 uL			200 uL		
12	75 uL			5 uL	10 uL		10 uL			200 uL		
13	65 uL			5 uL	10 uL		20 uL			200 uL		
14	55 uL			5 uL	10 uL		30 uL			200 uL		
15	75 uL			10 uL	10 uL		5 uL			200 uL		
16	70 uL			10 uL	10 uL		10 uL			200 uL		
17	60 uL			10 uL	10 uL		20 uL			200 uL		
18	50 uL			10 uL	10 uL		30 uL			200 uL		
19	70 uL			15 uL	10 uL		5 uL			200 uL		
20	65 uL			15 uL	10 uL		10 uL			200 uL		
21	55 uL			15 uL	10 uL		20 uL			200 uL		
22	45 uL			15 uL	10 uL		30 uL			200 uL		
23	80 uL			10 uL	10 uL					200 uL		
24	80 uL			10 uL	10 uL					200 uL		

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