Name:	**Mouse Serum**
Catalog Number:	**NMS**
Sizes Available:	**1.0 mL**
Concentration:	≥ 30.0 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Frozen Liquid
Activity:	≥ 1.0 CP50 Units/mL active classical pathway ≥ 40 AP50 Units/mL active alternative pathway Lot specific titers provided in the Certificate of Analysis
Buffer:	None
Preservative:	None, 0.22 µm filtered. Not certified as sterile.
Storage:	-70°C or below. Minimize freeze/thaw cycles.
Source:	Normal mouse blood.
Precautions:	Use normal precautions for handling animal products.
Origin:	Manufactured in the USA.

General Description: Normal mouse serum is tested for complement activity and certified to possess functional classical and alternative pathways of activation. NMS was prepared from mixed gender CD1 mice. Each sample of blood was collected without anticoagulants and, after coagulation, the liquid portion was separated by centrifugation. Serum was filtered through a 0.22 µm filter, aliqoted and frozen at -80°C.

Physical Characteristics & Structure: NMS is a clear, straw-colored liquid containing all proteins of normal mouse serum. Although the NMS is filtered through 0.22 µm sterile filters and is aliquoted into sterile containers, it is not packed under strictly sterile conditions and is therefore not certified as sterile.

Function: NMS is tested for classical pathway hemolytic activity using antibody-sensitized sheep erythrocytes (CompTech #B200) and for alternative pathway function using rabbit erythrocytes (CompTech #B300). The Certificate of Analysis provided with each lot gives a description of the assays and specific titers for the serum.

Assays: Mouse complement is famously difficult to assay (Ref 1, 2 & 3). Mouse serum rapidly loses its complement 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 2, 4 & 5) and a great many common mouse strains are genetically deficient in C5 (Ref 6 & 7) 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, normal mouse serum from CD1 mice exhibited a CH50 of only ~1 unit/mL in the assay system described below, however, with MCAR present the CH50 was ~8 units/mL . That is 8-fold higher complement titer with 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 also extremely unstable outside of the mouse. We have observed a rapid loss of complement activity after thawing (100% loss if left at 4^{o}C overnight). Thus, we advise rapidly thawing NMS and immediately using it in assays or if it is necessary aliquoting and freezing the NMS. It should be thawed rapidly in a water bath, moved immediately to wet ice when thawed, aliquoted and re-frozen as rapidly as possible. Upon use, thaw only when the experimental setup is ready for the NMS sample and keep it on wet ice after thawing.

In complement assays, every lot of mouse complement will have a different titer even from the same strain of mice. Preliminary assays will need to be done to determine the correct amount of NMS to use. Normal sensitized sheep cells (EA) work well if the lysis is boosted by MCAR. Most NMS samples generally contain a lot of hemoglobin and therefore 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 should be set up with buffers and MCAR at room temperature. Then EA (antibody-sensitized sheep erythrocytes (CompTech #B200)) are added, mixed and incubated 5 min at room temperature. 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 as this will result in inconsistent results. EA are used at 5 x 10^{8}/ml in GVB++ (CompTech #B100) and all dilutions are done in GVB++ buffer. 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. After 30 min at 37^{o}C, the assays are diluted with 200 uL cold GVBE (CompTech #B105), mixed by vortexing, centrifuged to pellet the unlysed cells and 200 uL of the supernatant should be transferred to a flat-bottomed microplate and the absorbance read at 415 nm. Alternatively a microcuvette and spectrophotometer may be used. Percent lysis is calculated by subtracting the background A415 values (average of #1 and 2) from the A415 vales for each of the experimental assays (e.g. #7-22) and dividing by the maximum lysis (average of #5 and 6) minus background and then multiplying by 100.

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		

Applications: NMS is used to provide a source of mouse complement for hemolytic assays. This NMS complement serum has been pre-tested and certified to exhibit fully functional classical and alternative pathway complement activation.

Precautions/Toxicity/Hazards: The source is mouse blood, therefore precautions appropriate for handling any animal blood-derived product must be used.
MSDS is available upon request.

References

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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