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| **Name:** | **Normal Human Serum** |
| **Catalog Number:** | **NHS** |
| **Sizes Available:** | 1.0 mL/vial |
| **Concentration:** | >55 mg protein/mL (see Certificate of Analysis for actual conc.) |
| **Form:** | Frozen liquid |
| **Activity:** | >90% active classical pathway<br>>90% active alternative pathway<br>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 human blood (shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II). |
| **Precautions:** | Use normal precautions for handling human blood products. |
| **Origin:** | Manufactured in the USA. |

**General Description**

Normal human serum is tested for complement activity and certified to possess fully functional classical and alternative pathways of activation. Our NHS is also referred to in our product descriptions as the NHS complement standard. A functional lectin pathway is assumed to be present, but standard assays for all of its initiating components (ficolins, MASPs, and MBL) have not been established. Therefore, we have not tested specifically for lectin pathway activity.

NHS was prepared from a large number of donors. Each unit of blood was collected without anticoagulants and each was individually tested for transfusion-transmissible diseases before pooling (see **Precautions/Toxicity/Hazards** below**)**. After coagulation, the liquid portion was separated by centrifugation. The different units were pooled, filtered through a 0.22 µm filter, aliqoted and frozen at -80°C.

All testing for complement activity was performed on the subsequently thawed samples to guarantee that the functional activity reported is what customers will receive when the samples are thawed. Complement activity is stable for several years if NHS is stored at -70°C or below continuously. A slight degradation of activity may be observed following additional freeze thaw cycles.

**Physical Characteristics**

NHS is a clear, straw-colored liquid containing all proteins of normal human serum. Although the NHS 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**

NHS 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 sera compared to a normal human serum standard.

**Assays**

The unit of classical pathway activity is the CH50. One CH50 unit is defined as the input of NHS complement standard yielding 50% lysis of $1x10^8$ EA (CompTech #B200) when incubated for 60 minutes at 37°C in a total reaction volume of 1.5 mL GVB++.

The unit of alternative pathway activity is the AP50. One AP50 unit is defined as the input of NHS complement standard yielding 50% lysis of $1.5x10^7$ rabbit erythrocytes (Er, CompTech #B300) when incubated for 30 minutes at 37°C in a total reaction volume of 100 µL GVB° containing a final Mg-EGTA concentration of 10 mM.

Complement assays have not been standardized due to the many variables involved in the performance of these activity titers. Many alternative methods are used in different laboratories (Law, S.K.A. and Reid, K.B.M. (1995); Morgan, B.P. ed. (2000); Dodds, A.W. and Sim, R.B. editors (1997)). Some of the variables are uncontrollable, for example, sheep erythrocytes vary from animal to animal and bleed to bleed, they lyse more easily as the cells age, and sheep erythrocytes drawn in the spring are more easily lysed that erythrocytes harvested in the fall. Different batches of sensitizing rabbit anti-sheep erythrocyte hemolysin give different titers. Erythrocytes settle during assays and different people use different re-mixing schedules (approximately every 5-10 min is recommended). These and other variables yield different titers for the exact same serum if assays are performed in different labs or even if they are performed in the same lab at different times. Comparison to a NHS complement standard allows some normalization. An international standard is under development, which will allow complement titers from different laboratories to be compared.

**Applications**

NHS is used to provide a source of complement for hemolytic assays. This NHS complement standard has been pre-tested and certified to exhibit fully functional classical and alternative pathway complement activation.

**Precautions/Toxicity/Hazards**

The source is human blood, therefore precautions appropriate for handling any blood-derived product must be used even though the source was shown by certified tests to be negative for HBsAg and for antibodies to HCV, HCV-NAT, STS, and HIV-1 and HIV-II.

Hazard Code: B WGK Germany 3

MSDS is available upon request.

**References**

Dodds, A.W. and Sim, R.B. editors (1997) Complement. A Practical Approach (ISBN 019963539) Oxford University Press, Oxford.

Law, S.K.A. and Reid, K.B.M. (1995) Complement 2nd Edition (ISBN 0199633568) Oxford University Press, Oxford.

Morgan, B.P. ed. (2000) Complement Methods and Protocols. (ISBN 0-89603-654-5) Humana Press, Inc., Totowa, New Jersey.

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