

## Plasma Derived Proteins and Enzymes

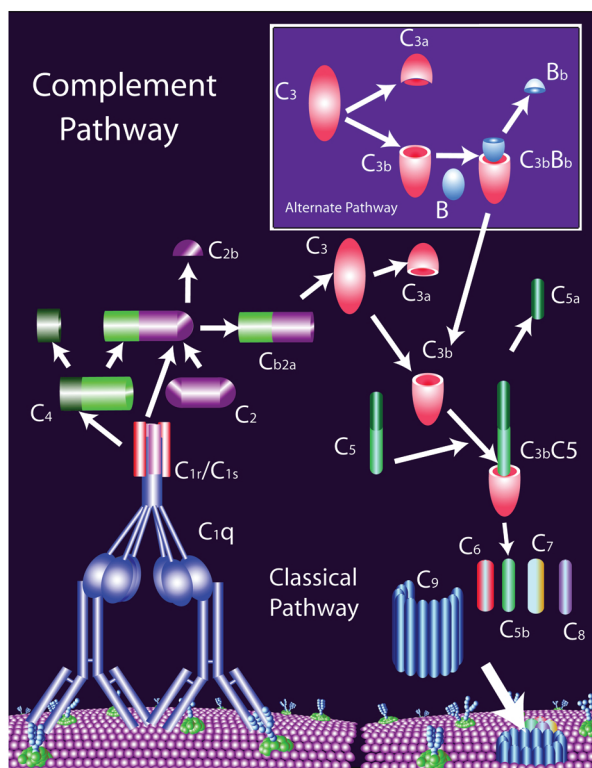
### Complement Proteins and Reagents

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The complement system is a complex cascade involving proteolytic cleavage of serum glycoproteins often activated by cell receptors. This cascade ultimately results in induction of the inflammatory response, phagocyte chemotaxis and opsonization, and cell lysis. Complement factors C3a, C5a, and C4 can induce vasodilatation, increased capillary permeability, and expression of leukocyte adhesion molecules. Complement factors C3a and C4a are opsonins that bridge phagocytes to microorganisms. Complement factors C3a and C4a promote phagocyte chemotaxis. Complement C3b may be an opsonin for antigen-antibody complexes which helps prevent damage from the formation of large, insoluble immune aggregates. Complement C5a, like C3a, is an anaphylatoxin and is a chemotactic attractant for induction of neutrophilic release of antimicrobial proteases and oxygen radicals. A complex of complements C5b, C6, C7, and C8 mediates the polymerization of up to eighteen C9 molecules into a tube-like membrane attack complex that is inserted into the plasma membrane of an unwanted organism such as Gram-negative bacteria and viral infected cells. This channel through the lipid bilayer results in lysis of the cell. Ischaemic infarction may also cause initiation of the complement cascade. Excessive deposits of membrane attack complexes in tissues may occur following ischaemic injury. Other effects of complement activation include, degranulation of neutrophils, basophils, and mast cells, release of the neutrophil products elastase and oxygen radicals, and extracorporeal blood circulation. Complement inhibitors are being studied as potential therapeutics for immune diseases and Alzheimer's.

**The Complement Pathways:** Three pathways have been elucidated through which the complement cascade can be initiated; Classical, Alternate and Lectin Pathways. All three pathways merge through at common intersection, complement C3. **The Classical Pathway:** The classical pathway mediates specific antibody responses. The classical pathway is initiated by the binding of antibodies to cell surface antigens. Subsequent binding of the antibody to complement C1q subunits of C1 result in catalytically active C1s subunits. The two activated C1s subunits are then able to catalyze the assembly of the C3 convertase (complement C4b2a) from complements C2 and C4. **The Alternate Pathway:** The alternate pathway does not require the action of antibodies to initiate the cascade, but is initiated by foreign cell surface components. In the alternate pathway complement C3 undergoes spontaneous cleavage resulting in complement B binding to C3b. Diffusion of the Ba subunit results in an active alternate pathway C3 convertase (C3bBb). C3bBb is stabilized by binding to properdin prior to merging into the common pathway and conversion of C3.

**The Lectin Pathway:** The lectin pathway is similar to the classical pathway. C1q is not involved in the lectin pathway. Instead an opsonin, mannan binding protein (MBP), is involved in the initiation process.



The Table lists the reagents needed to evaluate the hemolytic activities for each complement component and for whole complement serum. A detailed assay procedure is provided with each reagent.

Component	Antibody-sensitized sheep red blood cells (E9383)	GVB <sup>2+</sup> (G6514)	Deficient serum	Standard complement serum (C9473)
whole complement	2 vials	5 × 50 mL	—	1 vial
C2	1 vial	2 × 50 mL	1 vial <b>C0913</b>	1 vial
C3	1 vial	2 × 50 mL	1 vial <b>C8788</b>	1 vial
C4	1 vial	2 × 50 mL	1 vial <b>C1038</b>	1 vial
C5	1 vial	2 × 50 mL	1 vial <b>C1163</b>	1 vial
C6	1 vial	2 × 50 mL	1 vial <b>C1288</b>	1 vial
C7	1 vial	2 × 50 mL	1 vial <b>C1413</b>	1 vial
C8	1 vial	2 × 50 mL	1 vial <b>C1538</b>	1 vial
C9	1 vial	2 × 50 mL	1 vial <b>C1663</b>	1 vial

Our complement products are subjected to thorough quality control testing in our laboratories.

**Unit definitions:** The CH<sub>50</sub> unit for complement serum is defined as the volume (in mL) of serum that will result in a 50% hemolysis of 5 × 10<sup>8</sup> antibody-sensitized sheep red blood cells when incubated at 37 °C for 60 minutes in a final volume of 7.5 mL.

The H<sub>50</sub> unit for purified a component, or component in complement standard serum, is defined as the amount required to give a 50% hemolysis of 3 × 10<sup>7</sup> antibody-sensitized sheep red blood cells when incubated in the presence of the recommended volume of specifically deficient serum for 30 minutes at 37 °C in a final volume of 500 μL of the reaction mixture.

The AH<sub>50</sub> unit for the alternative pathway hemolytic activity of complement standard serum is defined as the quantity of serum required to yield 50% hemolysis of 1.5 × 10<sup>7</sup> rabbit red cells in one mL of reaction mixture containing 10 μL of 0.1 M MgEGTA (100 mM MgCl<sub>2</sub>, and 100 mM EGTA, pH 7.3) when incubated at 37 °C for 15 minutes.

**Hemolytic titer:** The number of CH<sub>50</sub> units in one mL of undiluted specimen. This is calculated as the reciprocal of the dilution which results in 50% hemolysis.

**Cytotoxic titer:** The NIH standard microlymphocytotoxicity technique is used to assay the sample for rabbit complement. It is defined as the greatest dilution which results in 80-100% cell death.

## Complement Proteins

### C1 Esterase Inhibitor from human plasma

Complement C1 esterase inhibitor; Esterase inhibitor C-1 from human plasma

[80295-38-1]

Esterase inhibitor C1 is a multifunctional regulator of all major kinin-generating protein cascade systems. It has been a therapeutic tool in the treatment of hereditary angioedema and complement-mediated inflammatory tissue damage such as capillary leak syndrome, septic shock, multiple organ failure, and hyperacute graft rejection.

mol wt ~105 kDa



#### ► aqueous solution, ≥90% (SDS-PAGE)

Functionally active.

Solution in phosphate buffered saline, pH 7.4, (15 mM sodium phosphate and 135 mM sodium chloride)

DRY ICE

C2412-.1MG 0.1 mg

#### ► aqueous solution, ≥95% (SDS-PAGE)

Solution in 20 mM potassium phosphate, pH 7.0, containing 250 mM KCl

DRY ICE

E0518-1MG 1 mg

### Complement C3 from human serum

[80295-41-6]

#### activity: ≥50 C3H<sub>50</sub> units/mg (using C3 deficient serum)

Complement C3 is the third and most abundant component of the complement pathway. It plays a central role in complement activation, being involved in both the classical and alternative pathways. C3 or its proteolytic fragments mediate many biological functions such as opsonization and anaphylatoxin activities. Pro-C3 is synthesized as a large single chain of 185 kDa and is processed to a disulfide-linked heterodimer consisting of an α-chain (115 kDa) and a β-chain (70 kDa) in the plasma. C3 is cleaved by the C3 convertases in either pathway (C4b2a, C3iBb, C3bBb) to C3b (176 kDa) comprised of the large C-terminal portion of the α-chain and the β-chain. C3b interacts with other complement components to initiate the amplification cascade. Functionally pure by a hemolytic assay using deficient sera.

Supplied as a solution in 15 mM sodium phosphate and 150 mM sodium chloride, pH 7.2.



DRY ICE

C2910-.1MG 0.1 mg

### Complement C4 from human serum

[80295-48-3]

#### activity: ≥300,000 C4H<sub>50</sub> units/mg protein

concentration .. 1 mg/mL in 25 mM sodium phosphate, 100 mM NaCl, pH 7.2, containing 0.02% sodium azide.



DRY ICE

C8195-.1MG 0.1 mg

### Complement C5 from human serum

[80295-53-0]

#### 75% (SDS-PAGE), activity: >300,000 C5H<sub>50</sub> units/mg protein

Functionally active by a sensitive hemolytic assay.

concentration ..... 1 mg/mL in 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2

DRY ICE

C3160-.1MG 0.1 mg

### Complement C5a human

C5a Anaphylatoxin; rC5a

[80295-54-1]

#### recombinant, expressed in *Escherichia coli*, ~95% (SDS-PAGE and HPLC), lyophilized powder

A mixture of C5a (~35%) and C5a having an added methionyl residue at the amino terminus (~65%); exhibits biological activities similar to serum-derived C5a. C5a is a (11.2 kDa) proteolytic fragment of the C5 α-chain through the action of C5 convertases in the classical and alternative complement pathway (C4b2a4b, C3bBb3b). C5a is an anaphylatoxin. It acts as an inflammatory chemoattractant. C5a stimulation of human neutrophils leads to STAT3 phosphorylation on Ser<sup>727</sup>. It mediates IL-8 release from bronchial epithelial cells. C5a anaphylatoxin activity on hepatocytes results indirectly from interaction with nonparenchymal cell via prostanoid secretion.

Mol. Wt.: ~8.6 kDa (non-glycosylated, with glutathione attached to cysteine 27).

◆ DRY ICE

C5788-.1MG 0.1 mg

C5788-.5MG 0.5 mg

### Complement Component 5 (C5a) Receptor human

CD88; Complement C5a

#### membrane suspension

Membranes suspended in 50 mM HEPES containing 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.1% bacitracin, and 0.1% bovine serum albumin, pH 7.3. Actual concentration and specific binding capacity are provided with each lot.

Volume of the suspension is 1.0 mL per vial.

DRY ICE

C9113-100UN 100 units

### Complement C6 from human serum

[80295-56-3]

#### 80% (SDS-PAGE), activity: >300,000 C6H<sub>50</sub> units/mg protein

Functionally active by a sensitive hemolytic assay.

concentration ..... 1 mg/mL in 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2

DRY ICE

C3285-.1MG 0.1 mg

### Complement C7 from human serum

[80295-57-4]

#### 60% (SDS-PAGE), activity: >200,000 C7H<sub>50</sub> units/mg protein

Functionally active by a sensitive hemolytic assay.

concentration ..... 1 mg/mL in 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2

DRY ICE

C2787-.1MG 0.1 mg

## Plasma Derived Proteins and Enzymes

### Complement Proteins and Reagents: *Complement Proteins*

#### Complement C8 from human serum

[80295-58-5]

**suitable for radioiodination, ≥85% (SDS-PAGE), activity:**

**≥125,000 C8H<sub>50</sub> units/mg protein (using C8 deficient serum)**

Functionally pure by a sensitive hemolytic assay using depleted sera. concentration .. 1 mg/mL in 15 mM sodium phosphate, 150 mM NaCl, pH 7.2

  DRY ICE



C3535-.1MG	0.1 mg
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#### Complement C9 from human serum

[80295-59-6]

**1 mg/mL in 15 mM sodium phosphate, 135 mM NaCl, pH 7.4, activity: ≥70,000 C9H<sub>50</sub> units/mg (using C9 deficient serum)**

Functionally pure by a sensitive hemolytic assay using deficient sera.

  DRY ICE

C3660-.1MG	0.1 mg
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#### Complement component C1q from human serum

[80295-33-6]

**solution (10 mM HEPES, pH 7.2. and 0.3 M NaCl), ≥95% (SDS-PAGE)**

C1q, together with C1r and C1s, in the ratio of 1:2:2, form the C1 complex which is the first component of the classical complement pathway. The C1q molecule is composed of 18 polypeptide chains (six A, six B, six C) (MW 460 kDa). All three types of chains contain an 81-aa collagen-like region composed of (Gly-Xaa-Yaa) repeating sequences close to the N-terminus. These three types of chains interact in sets of three (A, B, C) to form a triple helix with the C-terminus forming the globular heads which may be structurally and functionally distinct domains. One C1q molecule is composed of six triple helices.

  DRY ICE

C1740-.5MG	0.5 mg
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C1740-1MG	1 mg
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#### Complement factor D from human plasma

[37213-56-2]

**100 µg/mL in PBS, pH 7.2, >90% (SDS-PAGE)**

  DRY ICE

C5688-10UG	10 µg
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#### Complement factor H from human plasma

[80295-65-4]

**1 mg/mL in PBS, pH 7.2, >90% (SDS-PAGE)**

C3b-binding protein which regulates the formation and function of complement C3 and C5 convertases.

  DRY ICE

C5813-.1MG	0.1 mg
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#### Complement factor I from human plasma


[80295-66-5] E.C. 3.4.21.45

**1 mg/mL in PBS, pH 7.2, >90% (SDS-PAGE)**

Protease which cleaves and inactivates C3b and C4b

  DRY ICE

C5938-.1MG	0.1 mg
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 **Esterase inhibitor C-1**, see C1 Esterase Inhibitor Page 15

## Complement Sera


### ▼ Complement sera

Lyophilized powder from indicated amount of serum

#### Complement sera human

##### lyophilized powder

Hemolytic titer (CH<sub>50</sub> units per ml) is determined by method of Kabat and Mayer. Actual titer given on label.

 DRY ICE

S1764-1ML	1 mL
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
S1764-5X1ML	5 × 1 mL
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#### Complement Serum Standard human

##### aqueous solution

Substrate serum used as standard for quantitation of components C1q, C2, C3, C4, C5, C6, C7, C8, C9 and factor B.

Solution containing 10 mM EDTA


 DRY ICE

C9473-1ML	1 mL
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#### Complement sera from guinea pig

##### lyophilized powder

Hemolytic titer (CH<sub>50</sub> units per ml) is determined by method of Kabat and Mayer. Minimum 80 CH<sub>50</sub> units per mL. Actual titer given on label.

 DRY ICE

S1639-1ML	1 mL
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S1639-5ML	5 mL
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S1639-10X5ML	10 × 5 mL
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#### Complement sera from mouse

##### lyophilized powder

Hemolytic titer (CH<sub>50</sub>units per ml) is determined by the method of Kabat and Mayer. Actual titer given on the label.

 DRY ICE

S3269-1ML	1 mL
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#### Complement sera from rabbit

##### lyophilized powder

 DRY ICE


S7764-1ML	1 mL
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S7764-5ML	5 mL
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#### Complement sera from rat

##### lyophilized powder

Hemolytic titer (CH<sub>50</sub> units per mL) is determined by the method of Kabat and Mayer. Actual titer given on the label.

 DRY ICE

S3394-1ML	1 mL
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S3394-5X1ML	5 × 1 mL
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### Complement sera ▲

## Complement Deficient Sera

### Complement C2 deficient serum human

[80295-40-5]

For determination of complement C2 activity.

C2 is depleted by immunoabsorption method as judged by a highly sensitive hemolytic assay and Ouchterlony immunodiffusion method.



$-70^{\circ}\text{C}$  DRY ICE

C0913-1ML 1 mL

### Complement C3 deficient serum human

For the determination of complement C3 activity

C3 is depleted by immunoabsorption as judged by a highly sensitive hemolytic assay.



$-70^{\circ}\text{C}$  DRY ICE

C8788-1ML 1 mL

### Complement C4 deficient serum from guinea pig

C4 is deficient as judged by a highly sensitive hemolytic assay and Ouchterlony immunodiffusion method.



$-70^{\circ}\text{C}$  DRY ICE

C1038-1ML 1 mL

### Complement C5 deficient serum human

For determination of complement C5 activity

C5 is depleted by immunoabsorption as determined by hemolytic assay.



$-70^{\circ}\text{C}$

C1163-1ML 1 mL

### Complement C6 deficient serum human

For determination of complement C6 activity

C6 is depleted by immunoabsorption as judged by a highly sensitive hemolytic assay.



$-70^{\circ}\text{C}$  DRY ICE

C1288-1ML 1 mL

### Complement C7 deficient serum human

For determination of complement C7 activity

C7 is depleted by immunoabsorption as judged by a highly sensitive hemolytic assay.



$-70^{\circ}\text{C}$  DRY ICE

C1413-1ML 1 mL

### Complement C8 deficient serum human

For determination of complement C8 activity

C8 is depleted by immunoabsorption as judged by a highly sensitive hemolytic assay.



$-70^{\circ}\text{C}$

C1538-1ML 1 mL

### Complement C9 deficient serum human

For determination of complement C9 activity

C9 is depleted by immunoabsorption as judged by a highly sensitive hemolytic assay.



$-70^{\circ}\text{C}$  DRY ICE

C1663-1ML 1 mL

## Assay Reagents

### Antibody Sensitized Sheep Erythrocytes

EA7S

**$1 \times 10^9$  cells/mL, suitable for assay of complement component activity (H50 units) and whole complement activity (CH50 units), buffered aqueous cell suspension**

Suspension in gelatin veronal buffer containing 0.1 M sucrose

◆  $-2-8^{\circ}\text{C}$  WET ICE

E9383-2ML 2 mL

E9383-5X2ML 5 × 2 mL

### Gelatin veronal buffer

Gelatin Barbital Buffer; GVB<sup>2+</sup>

0.15 mM CaCl<sub>2</sub>, 141 mM NaCl, 0.5 mM MgCl<sub>2</sub>, 0.1% gelatin, 1.8 mM sodium barbital and 3.1 mM barbituric acid, pH 7.3-7.4.

Aseptically filtered

$-2-8^{\circ}\text{C}$

G6514-50ML 50 mL

G6514-5X50ML 5 × 50 mL

### Gelatin veronal buffer-EDTA

GVB-EDTA

sterile-filtered

Contains 141 mM NaCl, 0.1% gelatin, 1.8 mM sodium barbital, 3.1 mM barbituric acid, and 10 mM EDTA, pH 7.3-7.4.

$-2-8^{\circ}\text{C}$

G9660-50ML 50 mL

G9660-5X50ML 5 × 50 mL