TECOmedical Group

TECO® Mouse C3a ELISA

Mouse C3a ELISA

Instructions for use
English

Catalog No. TE1038
For Research Use Only

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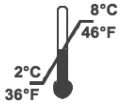
Symbol Description

Kit Instructions

Lot Number

Expiry Date

For Research Use only



Storage Temperature

Manufacturer

Caution: read instructions

TE1038

Attention

Intended use

Tests

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TECO® Mouse C3a ELISA Kit

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
1	**96-well plate coated with mouse C3a specific antibody with plate cover** 12 Break apart strips of 8 wells (12 x 8 in total), in a frame, ready for use	**1 plate**
S	**Standard Stock (lyophilized)** Conc. see data sheet	**2 vial**
L	**Control 1 (lyophilized)** Range as indicated on data sheet	**2 vial**
H	**Control 2 (lyophilized)** Range as indicated on data sheet	**2 vial**
2	**Wash Buffer** 50 times concentrated	**1 x 30 ml**
3	**Dilution Buffer** Ready for use	**1 x 60 ml**
4	**Matrix Solution** Ready for use	**1 x 6 ml**
5	**Biotinylated Antibody** Ready for use	**1 x 3 ml**
6	**SA-HRP Conjugate** Ready for use	**1 x 12 ml**
7	**TMB Substrate** Ready for use	**1 x 12 ml**
8	**Stop Solution – 1 M HCl** 1 M hydrochloric acid, ready for use 	**1 x 12 ml**
	**Kit instruction**	**1 x**

Storage

Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

Instructions for Use

The TECO® Mouse C3a kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of C3a in mouse serum, plasma and cell culture.

Intended Use

C3 is central to the classical, alternative and lectin pathways of complement activation.
The synthesis of C3 is tissue-specific and is modulated in response to a variety of stimulatory agents. During complement activation, C3 is proteolytically cleaved resulting in release of the anaphylatoxic peptide C3a.

C3a is a small polypeptide consisting of 74 amino acids. C3a itself is very short-lived and in serum cleaved rapidly into the more stable C3a-desArg (also called acylation stimulating protein, ASP). Therefore, measurement of C3a-desArg allows reliable conclusions about the level of complement activation in the samples.

Assay Principle

The TECO® Mouse C3a EIA Kit is a 96 well immuno-capture ELISA product. Samples are incubated with a specific monoclonal antibody coated on the plate. After incubation, the unbound material is washed away. Then a biotinylated monoclonal antibody that specifically recognizes mouse C3a is added to the wells. After a further incubation and washing step, a streptavidin-peroxidase conjugate is bound to the biotinylated polyclonal antibody, followed by incubation and washing.

TMB substrate is added which reacts with the enzyme and resulting in a concentration-depended color level. The reaction is stopped with HCl and the plate is read using a plate reader at 450 nm.

Color development is proportional to the amount of mouse C3a in the sample.

Materials Required and not Supplied

- Pipettes capable of accurately dispensing 10-1000 µl
- Multichannel pipette for 25-100 µl
- Graduated cylinders for reconstituting or diluting reagents
- Automatic washer or equivalent plate washing system
- Distilled or deionized water
- Vortex mixer
- ELISA plate shaker (orbital shaker, 500 rpm)
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm (Reference Filter: 590–650 nm).
- ELISA plate reader software for data generation and analysis

Warnings and Precautions

This kit is intended for in vitro research use by professional persons only.

Follow the instructions carefully.

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to "Materials Safety Data Sheet" for more detailed safety information.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat all specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any samples
3. Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
4. Disposal of containers and unused contents should be performed in accordance with federal and local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. Do not use coated strips if pouch is punctured.
8. It is recommended to test each sample in duplicate.
9. Use of multichannel pipettes is recommended to ensure the timely delivery of liquids, however, do NOT use a multichannel pipette for plate washing steps.
10. a) 1 M hydrochloric acid is caustic and can cause severe burns.
    b) Handle TMB (3,3',5,5'-tetramethylbenzidine) with care, and minimize exposure to light. Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.
11. As preservative 5-Bromo-5-nitro-1,3-dioxane (0,06 %) is used for the Wash Buffer, Biotinylated Antibody, Matrix Solution and Dilution Buffer. Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.

Preparation of Reagents

1 Microwell Plate Coated with Mouse C3a specific antibody

12 break apart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal. Store at 2–8 °C until expiration date.

S Standard

2 vials containing lyophilized mouse C3a.
See data sheet. Store at 2–8 °C until expiration date.

L Control 1

2 vials of lyophilized low control.
Range see data sheet. Store at 2–8 °C until expiration date.

H Control 2

2 vials of lyophilized high control.
Range see data sheet. Store at 2–8 °C until expiration date.

2 Wash Buffer

1 vial of 30 ml buffer, 50 x concentrated. Precipitation may occur in the buffer; resolve before use by warming up and mixing. Bring the vial content to 1500 ml with deionized or distilled water. The diluted washing solution is stable for 4 weeks at 2–8 °C.
Store undiluted buffer at 2–8 °C until expiration date.

3 Dilution Buffer

1 vial of 60 ml.
Ready for use. Store at 2–8 °C until expiration date.

4 Matrix Solution

1 vial of 6 ml.
Ready for use. Store at 2–8 °C until expiration date.

5 Biotinylated Antibody

1 vial of 3 ml.
Ready for use. Store at 2–8 °C until expiration date.

6 SA-HRP Conjugate

1 vial of 12 ml.
Ready for use. Store at 2–8 °C until expiration date.

7 TMB Substrate

1 vial of 12 ml of H_2O_2 and stabilized 3,3',5,5'-tetramethylbenzidine.
Ready for use. Store at 2–8 °C until expiration date.

8 Stop Solution – 1 M HCl

1 vial of 12 ml of 1 M hydrochloric acid.
Ready for use. Store at 2–8 °C until expiration date.

Preparation of Standard (in Dilution Buffer)

The Standard has to be prepared by using the lyophilized Standard Stock **S** . The volume for reconstitution of the stock standard, please see Certificate of Analysis provided with the kit. Reconstitute the lyophilized stock standard **S** . Let stand at room termperature for 25-35 minutes. Thereafter vortex vigorously and prepare standard curve by diluting Std. A 1:2 with dilution buffer according to the table below.

Note: Important that the lyophilized standard is completely reconstituted.

Discard reconstituted and diluted standards after use. Freezing of these standards is not possible.

ID	Concentration	Dilution Buffer
Std A	500 ng/mL	Preparation see data sheet (Certificate of Analysis)
Std B	250 ng/mL	250 µL Dilution Buffer + 250 µL Std A
Std C	125 ng/mL	250 µL Dilution Buffer + 250 µL Std B
Std D	62,5 ng/mL	250 µL Dilution Buffer + 250 µL Std C
Std E	31,25 ng/mL	250 µL Dilution Buffer + 250 µL Std D
Std F	0 ng/mL	Dilution Buffer

Preparation and Stability of Samples

Preparation of Controls

Add 250 µl Dilution Buffer **3** to the lyophilized Control 1 and Control 2. Let stand at room temperature for 25-35 min. Thereafter vortex vigorously and store the reconstituted controls in ice water until assayed. Discard reconstituted controls after use.

Note: Important that the lyophilized controls are completely reconstituted.

Sample Type and Preparation: Serum and Plasma

Non-lipemic samples are recommended. Centrifuge collected blood samples within 4 hours. Use immediately in the assay or keep frozen at - 80°C.

If samples are frozen, thaw with cold water and store samples in ice water until assayed.

Dilution of Samples

Pre-dilute samples e.g. 1 : 100 with Dilution Buffer **3** . To measure low concentration (< 3 µg/ml), dilute sample 1: 50 or 1: 25 with Dilution Buffer **3** . Lower dilution than 1: 10 are not recommended.

To measure concentrations expected higher than 50 mg/ml, sample should be used at a higher dilution (1: 200 or 1: 500).

Stability

Serum samples are not stable at room temperature. Longer storage at - 80°C in aliquots. Repeated freeze and thaw cycles should be avoided.

Assay Procedure

It is recommended that all determinations (Standards, Controls and samples) are assayed in duplicate. When performing the assay, Standards, Controls and samples should be pipetted as fast as possible (< 15 minutes). To avoid distortions due to differences in incubation times, Substrate Solution and Stop Solution should be added to the plate in the same order and with the same time interval.
Before use, allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes. During all incubation steps, plates should be sealed with the adhesive foil or a plastic cover. For light protection, incubate in a dark chamber or cover plate with aluminium foil.

1. Allocate the wells of the Microwell Plate **1** for Standards, Controls and samples.

2. Prepare the microassay strips as follows:
   Rehydrate microassay wells by adding approximately 300 µL of Wash Buffer **2** to each well using a wash device or automated filling device. Incubate for two minutes at 20–25°C. Remove the liquid from each well. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.

3. Add **50 µl** Matrix Solution **4** to each well (multichannel pipette).

4. Pipette **25 µl** of each Standard (Std. A - Std. F), reconst. Controls (**L** and **H**)
   and diluted samples into the corresponding wells.
   Note: Standards and Controls should not be further diluted.

5. Add **25 µl** of Biotinylated Antibody **5** (multichanel pipette).

6. Incubate the plate for **2 h** at room temperature (20–25 °C) on a shaker (500 rpm).

7. After incubation, aspirate the content of the wells and wash 3 times with 350 µl diluted Wash Buffer **2**.
   The use of an automatic plate washer is recommended.

8. Add **100 µl** of SA-HRP Conjugate **6** (multichannel pipette).

9. Incubate the plate for **30 min** at room temperature (20–25 °C) on a shaker (500 rpm).

10. After incubation, aspirate the content of the wells and wash 5 times with 350 µl diluted Wash Buffer **2** .
    The use of an automatic plate washer is recommended.

11. Pipette **100 µl** of the TMB Substrate Solution **7** in each well (multichannel pipette).

12. Incubate the plate for **15-30 min** in the dark at room temperature (20–25 °C) on a shaker (500 rpm).

13. Stop the reaction by adding **100 µl** of Stop Solution **8** (multichannel pipette).

14. Measure the color reaction within **10 min** at 450 nm (reference filter between 590–650 nm).

Result Analysis

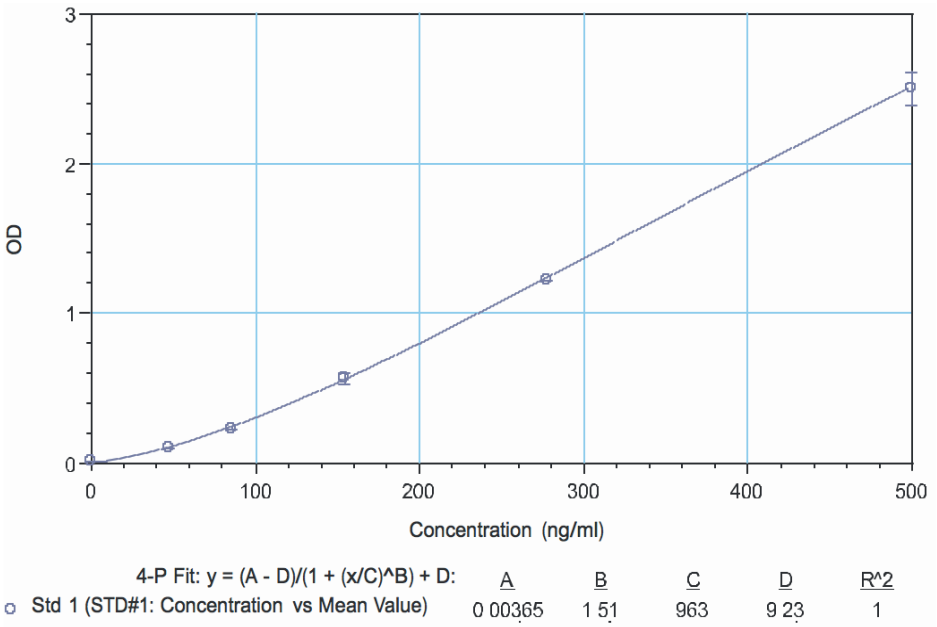
A standard curve can be established by plotting standard concentration on the x-axis (linear scale) against the absorbance of the standards on the y-axis (linear scale). A 4-parameter curve fit should be used for automatic data reduction. Alternatively, a quadratic (polynomial) fit is possible. The mouse C3a concentration of samples will be obtained by multiplying the value read off the standard curve by the dilution factor used for the given sample. For each assay, the results of the controls must be within the target range indicated for every lot. The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the provided range, the assay results should be considered questionable and the samples should be tested again.

Samples with higher absorbance values than Standard (Std. A) should be tested again with a higher dilution.

Typical Standard Curve and Controls

(Example only. Not for use in calculation of actual results)

Standards	Concentration (ng/ml)	Absorption at 450 nm
Standard (Std) A	500	2.632
Std B	250	1.413
Std C	125	0.645
Std D	62,5	0.254
Std E	31,25	0.121
Std F	0	0.014



Obtained Normal Values

5 - 20 µg/ml

Test Performance

Precision

Inter assay

Sample (serum)	n	Mean (mg/l)	CV (%)
Sample #1	20	104.2	3.6
Sample #2	19	365.6	3.6

Sample (serum)	n	Mean (mg/l)	CV (%)
Sample #1	11	105.0	2.8
Sample #2	11	361.4	4.3

Detection Limit

The kit detection limit was calculated in 20 Runs.
The mean detection limit is defined as Std. F (0 ng/ml) plus 3 SD: 4.5 ng/ml.

Spike Recovery

The recovery of mouse C3a spiked to normal samples was 97.4%.

Sample	Dilution	Measured ng/ml	Addition 115	Expected ng/ml	Recovery %
Sample #1	100	519	604	635	95
	200	315	415	430	96
	400	165	289	281	103
Sample #2	100	614	682	730	93
	200	364	460	479	96
	400	206	322	322	100
Sample #3	100	413	499	528	94
	200	228	337	343	98
	400	130	244	246	99
Sample #4	100	478	562	593	95
	200	279	380	394	96
	400	154	268	270	99
Sample #5	100	804	872	919	95
	200	494	604	610	99
	400	269	386	385	100
				Mean	**97.4**
				SD	**2.7**

Dilution Recovery

Sample	Dilution	Measured ng/ml	Expected ng/ml	Recovery %
Sample #1	100	> 500		
	200	315		
	400	165	157	105
Sample #2	100	> 500		
	200	364		
	400	206	182	113
Sample #3	100	413		
	200	228	206	110
	400	130	114	114
Sample #4	100	478		
	200	279	239	117
	400	154	139	111
Sample #5	100	> 500		
	200	494		
	400	269	247	109
			Mean	**111.4**
			SD	**3.9**

Species specifity

No cross reaction occurred with different species serum.

TECO® Mouse C3a ELISA

Assay Procedure – Quick Guide

- Bring reagents to room temperature.
- Washing Buffer: Dilute 1:50 with Aqua dest.
- Reconstitute Stock Standard **S** as mentioned in Certificate or Analysis and Controls with 250 µl Dilution Buffer.
- Thaw samples and mix well, and store on ice.
- Pre-dilute samples with Dilution Buffer **3** (e.g. 1: 100) and store on ice.

Prepare the required number of assay strips.

Pre-wash the plate by using **300 µl** Wash Buffer for **2 min** at 20-25°C.

Pipette **50 µl** Matrix solution **4** into each well (Multichannel pipette).
Add **25 µl** Standards (Std. A - Std. F), Controls (**L** and **H**) and diluted samples.
Add **25 µl** Biotin-AB **5** (Multichannel pipette).

Incubate **2 h** at 20-25 °C with shaking.

*Wash **3** times with Wash Buffer*

Pipette **100 µl** HRP Streptavidin Conjugate **6** into each well. (Multichannel pipette).

Incubate **30 min** at 20-25°C in the dark with shaking.

*Wash **5** times with Wash Buffer*

Pipette **100 µl** Substrate Solution **7** (Multichannel pipette).

Incubate **15-30 min** at 20-25°C in the dark with shaking.

Pipette **100 µl** Stop Solution **8** (Multichannel pipette).

Read the Optical Density at **450 nm** and using a reference filter between 590-650 nm.
Analyze the assay results using a 4- parameter curve fit: y= (A-D)/(1+(x/C)B)+D

Please read Kit instruction before using the Quick Guide