

**ELISA kits available from ADI (see details at the web site)**

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)		
#0020	Beta-2 microglobulin		
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M- 210-425-MC5

## Mouse Complement C5a ELISA Kit

### Cat # 210-425-MC5

**For Quantitative Determination of C5a in Mouse serum,  
plasma**

*For In Vitro Research Use Only*



4638 N Loop 1604 West • San Antonio • Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: [Techsupport@4adi.com](mailto:Techsupport@4adi.com)

Web Site: [www.4adi.com](http://www.4adi.com)

## Mouse Complement C5a ELISA KIT # 210-425-MC5

### Kit Contents (96 tests)

Components	#
<b>Anti- C5 coated</b> microwell strips (96 wells, 12 x 8 wells), # 425MC5-1P	1 Plate
<b>Standard Lyophilized</b> , # 425MC5-2	2 vials
Sample / Standard <b>dilution buffer</b> , # 425MC5-3, <b>20 ml</b>	1 bottle
<b>Biotin-detection antibody</b> (Concentrated), # 425MC5-4, <b>120 µl</b>	1 vial
Antibody <b>dilution buffer</b> , # 425MC5-5, <b>10 ml</b>	1 bottle
<b>HRP-Streptavidin Conjugate conc.</b> , # 425MC5-6, <b>120 µl</b>	1 vial
HRP-Streptavidin Conjugate <b>dilution buffer</b> , # 425MC5-7, <b>10 ml</b>	1 bottle
<b>TMB Substrate</b> , # 425MC5-TM, <b>10 ml</b>	1 bottle
<b>Stop Solution</b> , # 425MC5-ST, <b>10 ml</b>	1 bottle
<b>Wash buffer (25X)</b> , # 425MC5-WB, <b>30 ml</b>	1 bottle
Complete Instruction <b>Manual</b> , M-210-425-MC5	1

### Intended Use

ADI's **Mouse Complement C5a ELISA** kit is a sandwich type assay for the measurement of C5a in Mouse serum, plasma, tissue homogenates and other biological fluids. **For research use only (RUO)**, not for diagnostic procedures.

### General Information

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism. C5a is a protein fragment released from cleavage of complement component C5 by protease C5-convertase into C5a and C5b fragments. C5a is a chemotactic agent and an anaphylatoxin; it is essential in the innate immunity but it is also linked with the adaptive immunity. The increased production of C5a is connected with a number of inflammatory diseases. **Eculizumab** specifically binds to the terminal Complement component 5, and inhibits the cleavage of C5 to C5a and C5b by the C5 convertase, which prevents the generation of the terminal complement complex C5b-9 (which also has prothrombotic and proinflammatory effects). Both C5a and C5b-9 cause the terminal complement-mediated events that are characteristic of PNH and aHUS. In people with paroxysmal nocturnal hemoglobinuria (PNH), Soliris improves quality of life and decreases the need for blood transfusions. It is the first approved therapy for paroxysmal nocturnal hemoglobinuria. Eculizumab is also the first agent approved for treatment of atypical hemolytic uremic syndrome (aHUS).

For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, ADI shall have no liability.

4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## PERFORMANCE CHARACTERISTICS

### Precision

The within and between assay precision of the C5a ELISA Test System were determined by analyses on three 3 samples with low, middle and high level C5a.

CV (%) = SD/meanX100  
Intra-Assay: CV<8%  
Inter-Assay: CV<10%

### Sensitivity:

**Mouse Complement C5a ELISA Test System** has a sensitivity of **9.375 pg/ml**

**Detection Range:** 15.625- 1000 pg/ml

### Recovery

Matrices listed below were spiked with certain level of C5a and the recovery rates were calculated by comparing measured value to the expected amount of C5a in samples.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	85-104	97
EDTA plasma (n=5)	88-98	91
Heparin plasma (n=5)	85-103	90

### Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of C5a and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
Serum (n=5)	92-102%	87-98%	80-93%
EDTA plasma (n=5)	86-102%	84-97%	86-96%
Heparin plasma (n=5)	86-102%	86-99%	87-100%

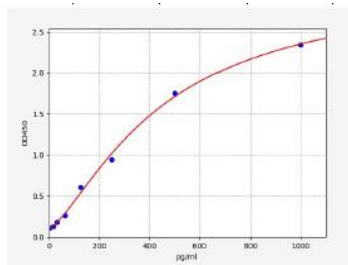
### Specificity

This assay has high sensitivity and excellent specificity for detection of C5a. No significant cross reactivity or interference between C5a and analogues was observed.

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm
A1, A2	Std. A (0 pg/ml)	0
B1, B2	Std. B (15.625 pg/ml)	0.021
C1, C2	Std. C (31.25 pg/ml)	0.072
D1, D2	Std. D (62.5 pg/ml)	0.154
E1, E2	Std. E (125 pg/ml)	0.499
F1, F2	Std. F (250 pg/ml)	0.834
G1, G2	Std. G (500 pg/ml)	1.642
H1, H2	Std. H (1000 pg/ml)	2.232

**NOTE:** These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



### Calculation of results:

A dose response curve is used to ascertain the concentration of C5a in unknown specimens.

- Record the A450 obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the A450 for mean duplicate calibrator versus the C5a concentration in ng/ml on linear graph paper. Connect the points with a best-fit curve.
- To determine the concentration of C5a for an unknown, locate the average A450 of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

### Interpretation

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.

## PRINCIPLE OF THE TEST

Mouse Complement C5a ELISA kit uses sandwich-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Anti- C5a antibody. And the biotin conjugated Anti- C5a antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution (stop solution) and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of C5a in the samples is then determined by comparing the OD of the samples to the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (50-200  $\mu$ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

## SPECIMEN COLLECTION AND STORAGE

**Plasma:** Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

**Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000xg. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

### Notes:

- Samples should be used within 7 days when stored at 2-8°C, otherwise samples must be divided and stored at -20°C ( $\leq$ 1month) or -80°C ( $\leq$ 6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
- Please take the samples to room temperature (18-25°C) without extra heating before performing the assay.
- Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

### Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from ADI's IFU may yield inaccurate results.
- All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
- It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

## REAGENTS PREPARATION FOR THE ASSAY:

**Wash Buffer (25X):** Dilute the wash buffer with distilled water (dissolve content of 1 bottle (30 ml) into 750 ml deionized or distilled water). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8°C.

**Standard:** 1) **1000 pg/ml of standard** solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

2) **500 pg/ml → 15.625 pg/ml** of standard solutions: **Label 6 Eppendorf tubes** with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.625pg/ml, respectively. Aliquot 0.3 ml of the Sample /Standard dilution buffer into each tube. Add 0.3 ml of the above 20 ng/ml standard solution into **1st tube** and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

**Note:** The standard solutions are best used within 2 hours. The standard solution should be at **4°C for up to 12 hours**. Or **store at -20 °C** for up to 48 hours. Avoid repeated freeze-thaw cycles.

**Biotin- labeled Antibody Working Solution:** Prepare it within 1 hour before experiment. Dilute the Biotin- labeled Antibody with Antibody Dilution Buffer at **1:100** and mix them thoroughly. (i.e. Add 1 µl of Biotin- labeled Antibody into 99 µl of Antibody Dilution Buffer)

**HRP-Streptavidin Conjugate Working Solution:** Prepare it within 30 minutes before experiment. Dilute the HRP-Streptavidin Conjugate with HRP-Streptavidin Conjugate dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of HRP-Streptavidin Conjugate into 99 µl of HRP-Streptavidin Conjugate dilution buffer)

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

## Quality Control:

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

**TEST PROCEDURE** (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE, 25-28°C, BEFORE USE). Prepare working solutions of biotin-antibody, HRP conjugate, Standard and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28°C).

1. Organize the microplates' wells for each standard, control and test sample to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. It's recommended to measure each standard and sample in duplicate. Wash plate 2 times with 350 ul wash buffer before adding standard, sample and control (blank) wells.
3. Pipette **100 µl of Mouse C5a standard, Blank or Sample (diluted at least ½ with sample/ standard dilution buffer)** into the assigned well. (The blank well is added with Sample/Standard dilution buffer). Seal the plate with a cover and incubate at 37 °C for 90 min.
4. Remove the cover and discard the plate content. Wash plate 2 times with 350 ul wash buffer. Do NOT let the wells completely dry at any time.
5. Add **100 µl of the Biotin- labeled antibody** to all wells.
6. Mix the microplate gently for 20-30 seconds by gentle tapping against the palm. Cover and **incubate for 60 minutes** at 37°C.
7. Remove the cover and Wash plate **3 times with 350 ul wash buffer**. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add **100 µl of HRP-Streptavidin Conjugate** to all wells. Mix the microplate gently for 20-30 seconds by tapping against the palm of your hand..
9. Cover and **incubate for 30 minutes** at 37°C.
10. Wash plate **5 times** with 350 ul wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
11. Add **90 µl of TMB substrate** reagent to all wells. Note: **Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION. Incubate at 37°C in dark within 15-30 minutes.**
12. Add **50 µl of stop solution** to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. **Read the absorbance in each well at 450nm. The results should be read within fifteen (15) minutes of adding the stop solution.**

**Note:** The matrix components in the sample will affect test results, which need to be diluted at least ½ with sample/ standard dilution buffer before testing.