Quantification of functional C3a using *iLite*® C3a Assay Ready Cells

For research and professional use only. Not for use in diagnostic procedures.

This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.

Background

Complement component 3a (C3a) is a 77 amino acid, 9 kDa, protein fragment of complement protein 3 (C3). C3 is highly abundant in the circulation and can be cleaved by C3 convertases to C3a and C3b as a result of complement activation. In analogy to C5a, C3a can also be generated by the extrinsic pathways, independently of the complement convertases, for example by factors of the coagulation and fibrinolytic pathways.
C3a induce effector functions by binding with the C-terminal to the C3a receptor, C3aR, a seven-transmembrane G protein-coupled receptor. Rapidly after C3a formation, if not bound to C3aR, the C-terminal arginine of C3a is removed by carboxypeptidases, resulting in C3a-desArg, that is unable to bind C3aR. (1, 2)

C3a are mainly described as a pro-inflammatory and chemotactic mediator, with weaker potency compared to C5a. Lately also anti-inflammatory characteristics has been described for C3a. Depending on cell type expressing C3aR and situation, C3a is able to induce either pro- or anti-inflammatory effects. This duality of C3a has been observed in several disease models of both acute and chronic inflammation. (3)

In the light of this duplexity, the therapeutic potential of targeting C3aR is still in research phase even though animal models have demonstrated the possible usefulness of both inhibition and activation of C3aR. Therefore, both agonists and antagonists of the C3a-C3aR axis may have potential therapeutic value. Several inhibitors of C3 has reached clinical trials while trials targeting C3a/C3aR are still lacking. (4)

The *iLite*®-platform offers a cell-based assay that enables functional studies of C3a, receptor C3aR and their interaction.

Principle of the assay

The *iLite*® C3a Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of a C3a responsive promoter and exhibits a dose-dependent increase in firefly luciferase (FL) reporter gene activity following treatment of cells with increasing concentrations of human C3a. (Fig.1). The cells also contain the Renilla luciferase (RL) internal standardization gene under the control of a constitutive promoter that renders assay results independent of cell number and provides a means for correcting for cytotoxic effects that may be encountered with some biological samples.
The luciferase signals can be measured in a luminometer following addition and incubation of luciferase substrate.

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Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® C3a Assay Ready Cells	Svar Life Science	BM4073
Diluent (DMEM containing 1% Penicillin-Streptomycin)	Gibco	31966-021 (DMEM) 15140-122 (Penicillin-Streptomycin)
C3a or analogues	Complement Technologies	A118
Firefly/Renilla luciferase substrate	Promega	E2920, Dual-Glo Luciferase Assay System
Plate; White walled micro well plate suitable for luminescence	Revvity	6055680
Microplate Luminometer with appropriate reading software – no filter on luminometer	Contact Svar Life Science for list of recommended suppliers	NA
Incubator, 37 °C with 5% CO_2	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA
Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of calibrators (C3a)

Native human C3a, purified from human serum, from Complement Technologies has successfully been used to stimulate the *iLite*® C3a Assay Ready Cells. The below table shows the dilutions of C3a, used for QC release of the *iLite*® C3a Assay Ready Cells.

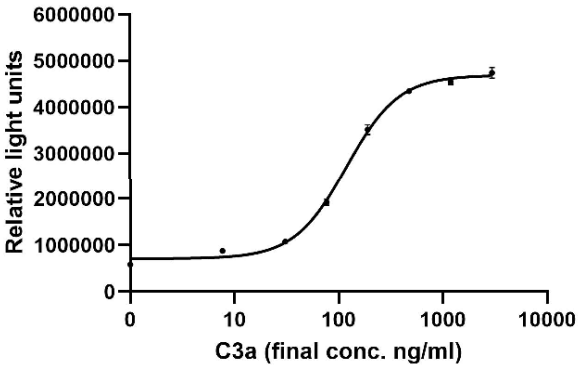

Figure 1. Example of C3a calibration curve.

Calibrator	C3a
	Suggested calibrator **solution conc**. (ng/ml)
A	6 000
B	2 400
C	960
D	384
E	154
F	61
G	15
H	0

Table 1. Suggested calibrator **solution concentrations** for C3a.

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Dilute calibrators, controls, and samples to fall within the expected **in assay concentrations** of 0 – 3 000 ng/mL.
3. Add 40 µL calibrators, controls, and samples in duplicate to assigned wells (final concentration will be half of solution concentration).
4. Thaw the vial of *iLite*® C3a Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette to ensure a homogeneous distribution of cells.
5. Dilute 250 µL cell suspension with 5.75 mL Diluent.
6. Add 40 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 5 hours at 37 °C with 5% CO_2.

Adding substrate solutions

1. Equilibrate the plate and the substrate solution to room temperature.
2. Prepare the **Firefly luciferase** substrate according to the manufacturer's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.
3. If appropriate, prepare the **Renilla luciferase** substrate according to the manufacturer's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.

Precautions

- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the manufacturer's instructions or product specifications accompanying the individual material and instruments.
- Dispose of all sample specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as though potentially infectious.
- Residues of chemicals and preparations are generally considered as biohazardous waste and should be inactivated prior to disposal by autoclaving or using bleach. All such materials should be disposed of in accordance with established safety procedures.

Proprietary Information

In accepting delivery of *iLite*® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third-party recipient, and only to use them directly in assays. *iLite*® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered *iLite*® Assay Ready Cells is an infringement of these patents.

QUICK GUIDE

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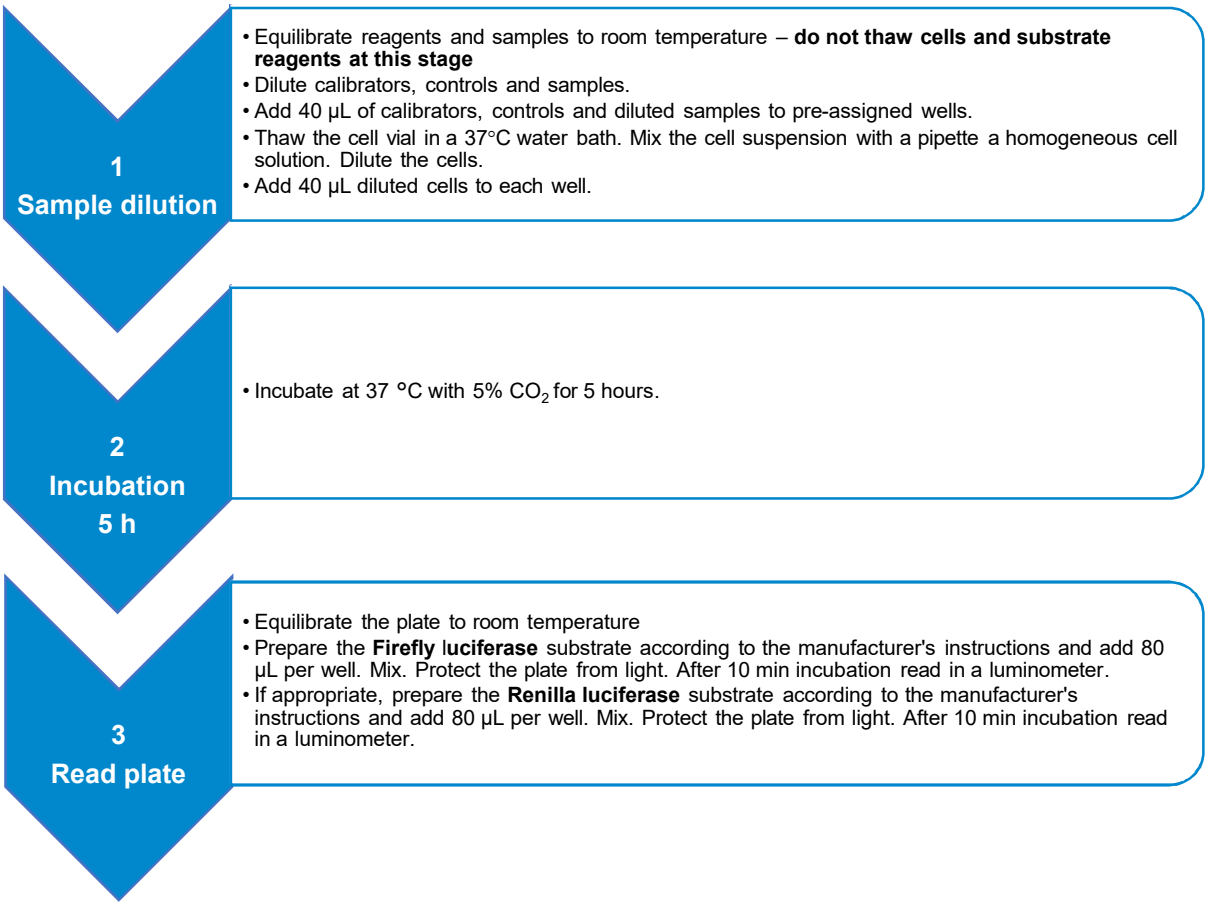

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com

References

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2. Gao et al. The Complement C3a and C3a Receptor Pathway in Kidney Diseases. Front Immunol. 2020 Aug 18; 11:1875. doi.org/10.3389/fimmu.2020.01875.
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