iLite® ADCC Activity Assays

ASSAY READY CELLS

***iLite*® ADCC Activity Assays are designed to enable determination of the ADCC activity of existing or new drug candidates.**

Measuring the ability of a drug to induce antibody dependent cell mediated cytotoxicity (ADCC) activity in a straight forward, sensitive and reproducible manner is a key aspect in development of antibody-based therapeutics.

The ***iLite* ADCC Activity Assay**, unique in its setup, comprises of **ADCC Effector Assay Ready Cells and matched Target Assay Ready Cells**, engineered to work optimally together and give unparalleled sensitivity when assessing ADCC activity even in complex matrix.

A negative control cell line is also available for each target, allowing screening for unspecific activation of ADCC, giving added value to each experiment.

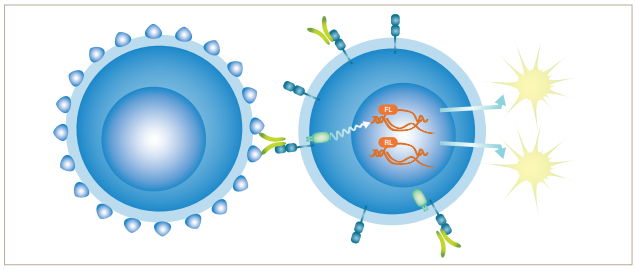


Figure 1. *The interaction between the reporter gene effector cell and the target cell, generated by the crosslinking of the two cells via a specific drug antibody. The resulting luminescence originates exclusively from this crosslinking and the signaling from the CD16 receptor to the Firefly Luciferase promoter. The strength of the luminescence correlates to the drugs ability to induce ADCC.*

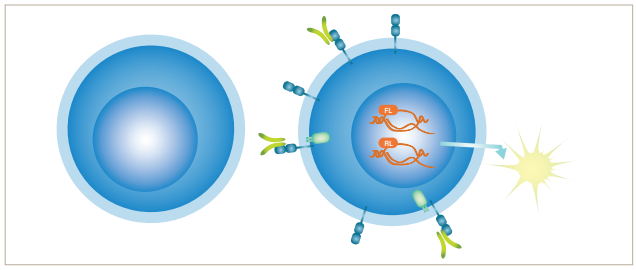
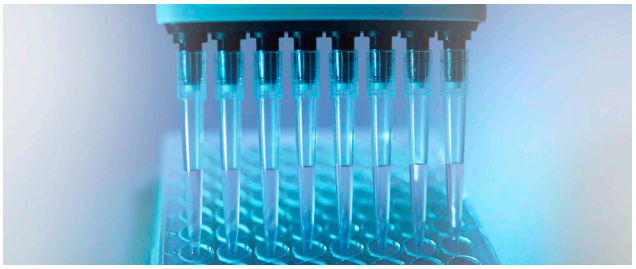


Figure 2. *When the target cell has been depleted of the specific surface target molecule, the crosslinking between the effector and the target cell cannot be established and hence no luminescence from Firefly Luciferase is generated. However, luminescence from the normalization gene Renilla, which is expressed under a constitutive promotor, is still present.*

As the majority of the *iLite* cell lines, the *iLite* ADCC Effector (V) Assay Ready cells also have a secondary luciferase readout, from a luciferase generated under the control of a constitutive promotor. This enables normalization of each individual read out according to cell number and thereby, accounting for any potential matrix effects.

Since the activity of many antibody-based therapeutics is mediated in part by their ability to induce ADCC and thereby enhancing the body's own immune response towards dysfunctional cells, it is a key parameter to analyze. By using the *iLite* ADCC Activity Assay when establishing the ADCC activity induced by different drugs or drug candidates, a measure of efficacy and safety can be provided.



iLite® ADCC Activity Set
General Product set up

- *iLite*® ADCC Effector Assay Ready Cells
- *iLite*® ADCC Target (+) Assay Ready Cells
- *iLite*® ADCC Target (-) Assay Ready Cells

***iLite*® ADCC Activity Assay Sets**	
BM5070	*iLite*® anti-CD20 ADCC Activity Set
BM5080	*iLite*® anti-EGFR ADCC Activity Set
BM5090	*iLite*® anti-HER2 ADCC Activity Set
BM5095	*iLite*® anti-mTNF-alpha ADCC Activity Set

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

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, and recipient is only to use them directly in assays. The 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 would constitute an infringement.

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Answers in Life Science

Doc No: E-291-GB00, September 2018

A novel method for quantification of ADCC activity based on the use of engineered effector cells and a series of matching target cells

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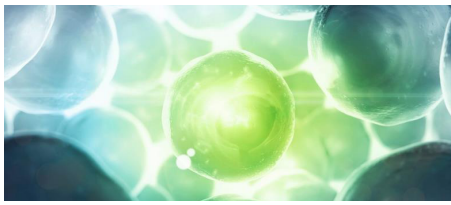

Introduction

The activity of numerous therapeutic antibodies is mediated in part by antibody-dependent cell-mediated cytotoxicity (ADCC). Traditional methods for quantifying ADCC activity are labor intensive and have a high level of inherent variability due to the use of primary human NK-cells from different donors as the effector cells. These limitations can be overcome in part by the use of an engineered effector cell line expressing the low affinity Fc receptor, FcgRIIIa (CD16), that responds to ligation of the Fc moiety of an antibody bound to the specific antigen expressed on target cells by activation of a NFAT responsive reporter gene. There is a need, however, for an ADCC assay with improved sensitivity, specificity, and tolerance to the presence of human serum.

Here we present a method for quantification of ADCC activity based on the use of novel engineered effector cells carrying a reporter gene. In addition, novel target cells have been developed that express a constant high level of the specific antigen as well as the homologous control target cells which allows differences in ADCC activity to be determined with precision and a high degree of specificity.

ADCC Reporter Gene Concept

Antibody dependent cell mediated cytotoxicity (ADCC) is the lysis (killing) of an antibody coated **target cell** by a cytotoxic **effector cell** through a nonphagocytic process, characterized by the release of the content of cytotoxic granules or by the expression of cell death inducing molecules. ADCC is triggered through interaction of target bound antibodies with certain Fc receptors (FcRs) present on the effector cell surface that bind the Fc region of the antibodies.

In the ***iLite*® ADCC Cell Line** the effector cells serves as the "killing cell". This means that, when activated, the antigen on the targets cell surface binds to the drug/antibody and in turn the FC receptor on the target cells, the reporter gene construct is activated and firefly luciferase produced (Fig. 1).

Establishment of an Engineered Effector Cell Line and Target Cells

For the effector cells - Jurkat cells were co-transfected with a chimeric promoter containing binding sites for the principal transcription factors (NFAT, NFkB, AP1, CREB, and STAT) that mediate signaling from the FcgRIIIa receptor, driving transcription of the firefly luciferase (FL) reporter-gene from a minimal SV40 promoter, an expression vector for FcRgIIIa (v variant), and the Renilla luciferase (RL) reporter gene, under the control of a constitutive promoter, that allows drug-induced FL activity to be normalized with respect to the constitutive expression of RL.

For the target cells - The target cells have been engineered to over-express a constant high level of the specific antigen recognized by the therapeutic antibody, and homologous control cells have been developed in which the gene encoding the specific drug target has been invalidated by CrisPR/Cas9 editing.

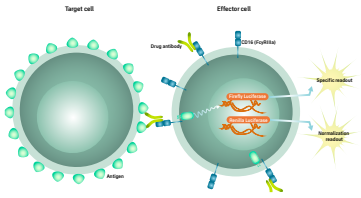

Figure 1. Schematic illustration of the *iLite* Reporter gene ADCC Effector cell.

Briefly the gene encoding for the target is invalidated using CRISPR/Cas9 genome editing, creating a target negative cell. These target negative cells were then transfected with a target expression vector and positive, stable clones were isolated and characterized for ADCC activity in the presence of *iLite* effector cells and drug/antibody. Overexpression of the surface antigen results in a high constant level of antigen at the surface, yielding a significant higher ADCC activity compared to wild type target cells.

Results

Quantification of ADCC activity of Trastuzumab together with HER2 (+) and (-) target cells as well as a comparison against a traditional NFAT effector cells and Wild type target cells (SK-BR3)

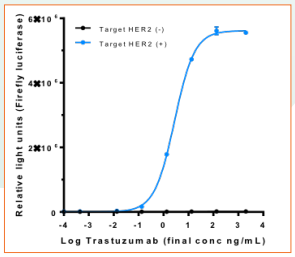

Figure 2.
iLite ADCC effector cells were assayed together with *iLite* target cells HER2 positive and negative and Trastuzumab. The positive target cells give a clear dose-response curve, whereas the negative target minus cells gives no response.

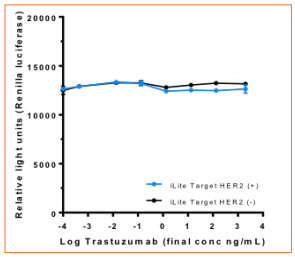

Figure 3.
The Renilla readout, from normalization gene, with the same set up as Fig. 2 show a very similar readout from the positive and negative target cell, due to the independent constitutos promotor region controlling the Renilla luciferase

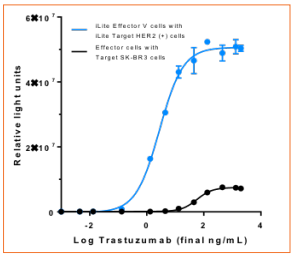

Figure 4.
The ADDC activity of Trastuzumab with the *iLite* ADCC system (effector cell and HER2 (+) target cells compared to a traditional NFAT effector cell and Wild type target cells. (SK-BR3).

Quantification of ADCC activity of cetuximab together with EGFR (+) and (-) target cells as well as a comparison against a traditional NFAT effector cells and Wild type target cells (A431)

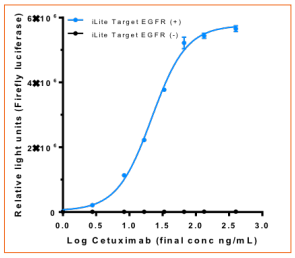

Figure 5.
iLite ADCC effector cells were assayed together with *iLite* target cells EGFR positive and negative and Cetuximab. The positive target cells give a clear dose-response curve, whereas the negative target minus cells gives no response.

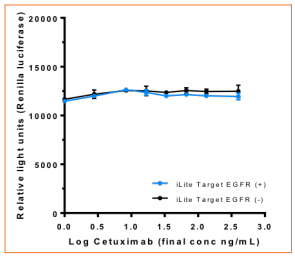

Figure 6.
The Renilla readout, from normalization gene, with the same set up as Fig 5. show a very similar readout from the positive and negative target cell, due to the independent constitutos promotor region controlling the Renilla luciferase translation.

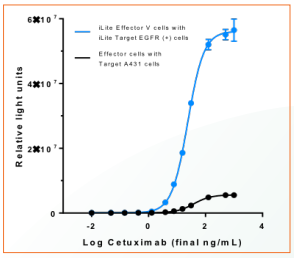

Figure 7.
The ADDC activity of Cetuximab with the *iLite* ADCC system (effector cell and EGFR (+) target cells compared to a traditional NFAT effector cell and Wild type target cells (A431).

Quantification of the ADCC Activity of Trastuzumab and Cetuximab resp. in the presence of Normal Human Serum

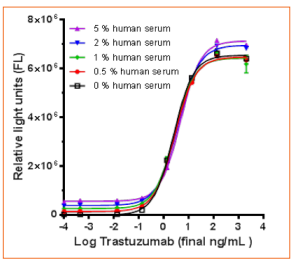

Figure 8.
The response of effector cells and HER2 (+) target cells upon activation with drug (trastuzumab) was visible in the presence of human serum.

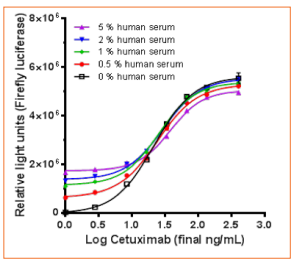

Figure 9.
The response of effector cells and EGFR (+) target cells upon activation with drug (cetuximab) was visible in the presence of human serum.

Note:

In accordance with the results from HER2 and EGFR, iLite ADCC effector cells were assayed together with iLite target cells mTNFα positive and negative and Infliximab and target CD20 (+/-) and Rituximab, with a very similar results (data not shown).

Conclusion

The effector cell lines described herein express Firefly luciferase activity in response to ligation of an antibody to the V-variant of CD16a and also express the Renilla luciferase reporter gene under the control of a constitutive promoter.

A series of novel target cells has been developed that expresses a constant high level of the specific antigen, recognizable by therapeutic antibodies, under the control of a strong constitutive promoter as well as the homologous control target cells in which the gene encoding the specific antigen has been inactivated by CRISPR/Cas 9 genome editing.

These engineered target cells and the homologous control cells allow differences in ADCC activity to be determined with precision. The expression of a constant high level of a specific antigen on the surface of the engineered target cells was associated with increased ADCC activity in the presence of the *iLite* effector cells and a specific therapeutic antibody, relative to that observed with a traditional NFAT effector cells and wild type target cells.

The availability of target cells specific for several of the most widely used therapeutic antibodies known to exhibit ADCC activity provides a precise means of comparing the ADCC activity of biosimilars with that of the innovator product as well as the comparison of the ADCC activity of variants of novel therapeutic antibodies that target the same antigen. The availability of target cells in which the specific drug target has been invalidated by genome editing provides the ideal control target cell for determining the specificity of an ADCC assay.

Furthermore, the improved tolerance to the presence of human serum and the presence of the luciferase normalization gene provide means for compensating serum matrix effects and killing of the effector cells by the target cells observed at high concentrations of antibody or in the presence of certain clinical samples.