iLite® ADCC CD20

ASSAY READY CELLS

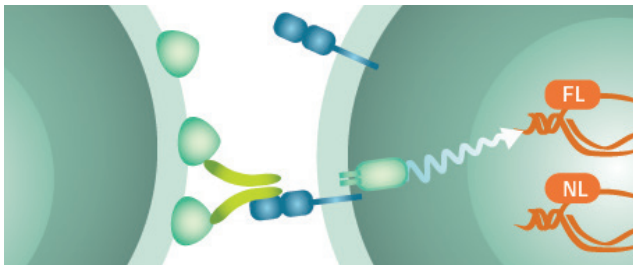
***iLite* ADCC anti-CD20 Activity Assay is designed to enable determination of the ADCC activity of existing or new anti-CD20 drug candidates.**

The idea of employing ADCC (antibody-dependent cell-mediated cytotoxicity) to destroy dysfunctional cells by treating patients with antibodies has existed since the discovery of the ADCC mechanism. Rituximab, one of the first of such drugs approved, is a chimeric monoclonal antibody targeting CD20, a surface antigen primarily found on B-cells.

The ***iLite* ADCC anti-CD20 Assay Ready Cells** are genetically engineered cell lines, optimized and matched to give high sensitivity and specificity in an anti-CD20 ADCC activity assay which can be run within one workday and does not require any cell culturing.

***iLite* ADCC Effector (V) Assay Ready Cells** are human cells which express high levels of FcγRIIIa (CD16) and signals to a Firefly Luciferase (FL) reporter gene. The cells are engineered to have a high tolerance for serum, and include a second reporter gene, NanoLuc® (NL), which allows for normalization of cell counts, serum matrix effects or lysis of the effector cells by the target cells.

The ***iLite* ADCC Target CD20 (+) Assay Ready Cells** are human cells engineered to overexpress CD20 and optimized to give high sensitivity and specificity when used together with ***iLite* ADCC Effector (V) Assay Ready Cells**. Since unspecific activation of ADCC can be a confounding factor when performing ADCC assays, we have also developed ***iLite* ADCC Target CD20 (-) Assay Ready Cells** which are depleted of CD20 expression, to be used as a control.



The iLite anti-CD20 ADCC Activity Set can be used for the quantification of ADCC activity of anti-CD20 antibodies

- Unparalleled sensitivity (EC50 of 2 ng/mL)
- High serum tolerance (no effect at 10%)
- Normalization readout
- High precision, due to Assay Ready Target cells with high CD20 expression.

***iLite*® ADCC CD20 Assay Ready Cells**	
Product code	BM4001 *iLite*® ADCC Effector (V) Assay Ready Cells BM4010 *iLite*® ADCC Target CD20 (+) Assay Ready Cells BM4015 *iLite*® ADCC Target CD20 (-) Assay Ready Cells BM4070 *iLite*® anti-CD20 ADCC Activity Set
Host Cell	BM4001: Human T lymphocyte cell line, Jurkat (ATCC #TIB-152) BM4010, BM4015: Human B lymphocyte cell line, Raji (ATCC# CCL-86)
Format	Assay Ready Cells
Application	The *iLite*® ADCC Effector (V) Assay Ready Cells can be used together with matched iLite® ADCC Target CD20 (+) and iLite® ADCC Target CD20 (-) Assay Ready Cells for the quantification ADCC activity. • Quantification of anti-CD20 ADCC activity (E-229-GB)
Assay time	30 min + 4 hours (incubation) + 30 min
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**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 Euro Diagnostica AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.

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Quantification of the ADCC activity of therapeutic antibodies

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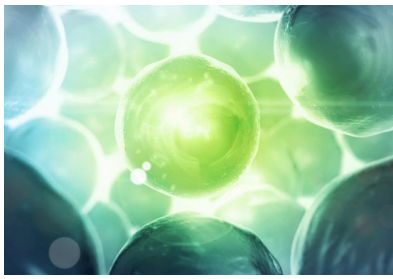


Introduction

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 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.

Results

I. Establishment of an Engineered Effector Cell Line

In order to establish a reporter-gene construct that responds optimally to ligation of the FcgRIIIa receptor (CD16),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 (Figure 1), an expression vector for FcRgIIIa (v variant), and the NL 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 NL (Figure 2), rendering assay results independent of variations in cell number, serum matrix effects, or lysis of the effector cells by the target cells.

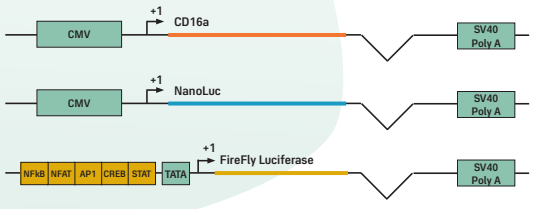
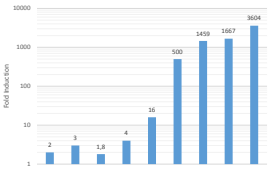


Figure 1. *Genetic constructs*

The use of this novel effector cell line confers improved sensitivity, dynamic range, tolerance to human serum, and a reduced incubation time, relative to engineered effector cell lines that express a NFAT regulated reporter-gene, when used in an ADCC assay together target cells that have been engineered to over-express a constant high level of the specific antigen recognized by the therapeutic antibody, and homologous control cells in which the gene encoding the specific drug target has been invalidated by CrisPR/Cas9 genomic editing.

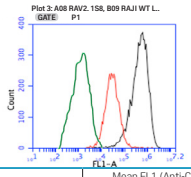


STAT (IFN γ/α 100 IU/ml)	X				X		X		X
NFkB (TNFα 100ng/ml)		X			X			X	X
CREB (dBcAMP 10mM)			X			X	X	X	X
NFAT (Ionomycin 500nM)				X		X	X	X	X
AP1 (PMA 10ng/ml)						X	X	X	X

Figure 2. *Synergistic activation of the ADCC-Luc reporter construct*

II-1: Establishment of an Engineered Target Cell Line Expressing High Constant Levels of CD20 at the Cell Surface

The gene encoding CD20 was invalidated in the B-cell line Raji (ATCC® CCL-86) using CrisPR-Cas9 genome editing. CD20 -/- Raji cells were then transfected with a CD20 expression vector and stable clones were isolated and characterized for CD20 expression (Figure 3) and ADCC activity in the presence of JE5.35 effector cells and rituximab and then sub-cloned.



Cell line	Mean FL1 (Anti-CD20-FITC)
RAJI CD20 -/-	1842
RAJI WT	36 704
RAJI CD20+/+	602 342

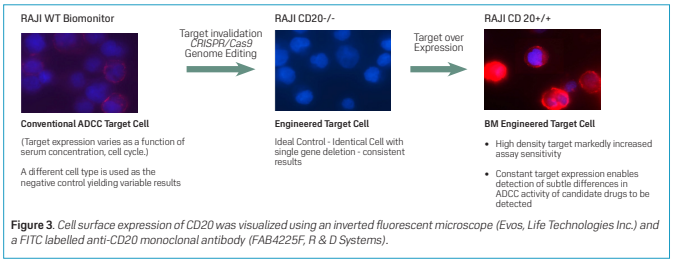


Figure 3. *Cell surface expression of CD20 was visualized using an inverted fluorescent microscope (Evos, Life Technologies Inc.) and a FITC labelled anti-CD20 monoclonal antibody (FAB4225F, R & D Systems).*

II-2: Establishment of the Optimal E:T Ratio

A fixed concentration of effector cells (E) was incubated with varying concentrations of CD20++ target cells (T) in order to determine the optimal effector target cell ratio (E:T) for the quantification of the ADCC activity of rituximab (Figures 4A & 4B). The optimal E:T ratio was found to be 3:1 (Figures 4A & 4B) after 4 hours incubation in the presence of increasing concentrations of rituximab. The level of Nano-Luc expression did not increase as a function of the E:T ratio (Figure 4C) and can thus be used as a normalization gene.

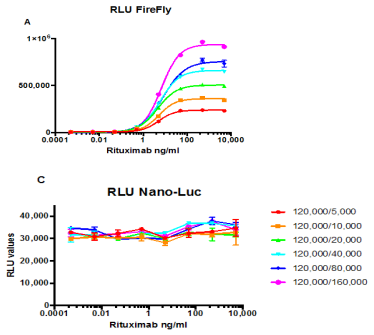


Figure 4.
Quantification of the ADCC Activity of Rituximab using JE5.35 Effector Cells & CD20++

Target Cells: Determination of the Optimal E:T ratio

III. Quantification of the ADCC Activity of Rituximab using Frozen Ready-to-Use Cells

In addition to providing a convenient and cost-effective means of quantifying the ADCC activity of therapeutic antibodies, frozen ready-to-use effector and target cells also provide the basis for the establishment of highly precise and reproducible assays with a low degree of vial-to-vial and lot-to-lot variation as illustrated in Figure 5.

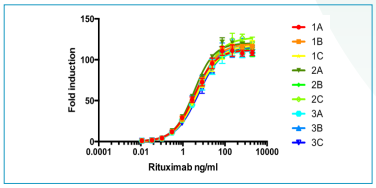
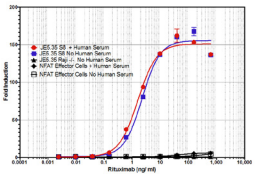


Figure 5.
Quantification of the ADCC Activity of Rituximab using Frozen Ready-to-Use Cells (Fold Induction, 4 hours)

IV. Quantification of the ADCC Activity of Rituximab in the presence of Normal Human Serum

The response of JE5.35 effector cells & CD20++ target cells was significantly greater than that of the NFAT effector cells & wild type Raji cells both in the presence and absence of human serum (Figure 6). Thus, a dynamic range of approximately 150 fold and an EC50 of 2.0 ng/ml was obtained for the JE5.35 effector cells & CD20++ target cells versus a dynamic range of 10 fold and an EC50 of 1.2 mg/ml for the NFAT/WT Raji cell assay in the absence of human serum. The JE5.35/CD20++ assay was markedly less affected by the presence of human serum (10 % final concentration) compared to the NFAT/WT Raji assay (Figure 6). The response of JE5.35/Raji -/- target cells was unaffected by increasing concentrations of rituximab (Figure 6).



Best-fit values	JE5.35 S8 Human Serum	JE5.35 S8 No Human Serum	JE5.35 Raji -/- No Human Serum	NFAT Effector Cells Human Serum	NFAT Effector Cells No Human Serum
Top	151,3	155,7	1,102	6,354	12,11
Bottom	0,9549	1,492	1,016	1,136	1,175
LogIC50	0,201	0,3517	−1,582	1,66	3,11
HillSlope	1,295	1,356	−12,82	1,204	0,8762
IC50	1,589	2,247	−38,19	45,72	1289
Span	150,3	154,2	0,08582	5,218	10,93

Figure 6. *Quantification of the ADCC Activity of Rituximab in the presence of Normal Human Serum*

Conclusions

The *iLite* effector cell line JFRN5.35 provides a highly sensitive, precise, and specific means of quantifying ADCC activity. The availability of both frozen ready-to-use effector and target cells, provides a convenient and cost-effective means of quantifying the ADCC activity of therapeutic antibodies, and also provides precise and reproducible assays with a low degree of vial-to-vial and lot-to-lot variation. The *iLite* effector cell line and specific target cells and the homologous target negative control cells can be used for both a potency assay and for the quantification of ADDC activity in clinical studies due to both the improved tolerance to human serum and the Nano-Luc normalization gene that provides a means for compensating for serum matrix effects, or killing of the effector cells by the target cells observed at high concentration of antibody in certain clinical samples.

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