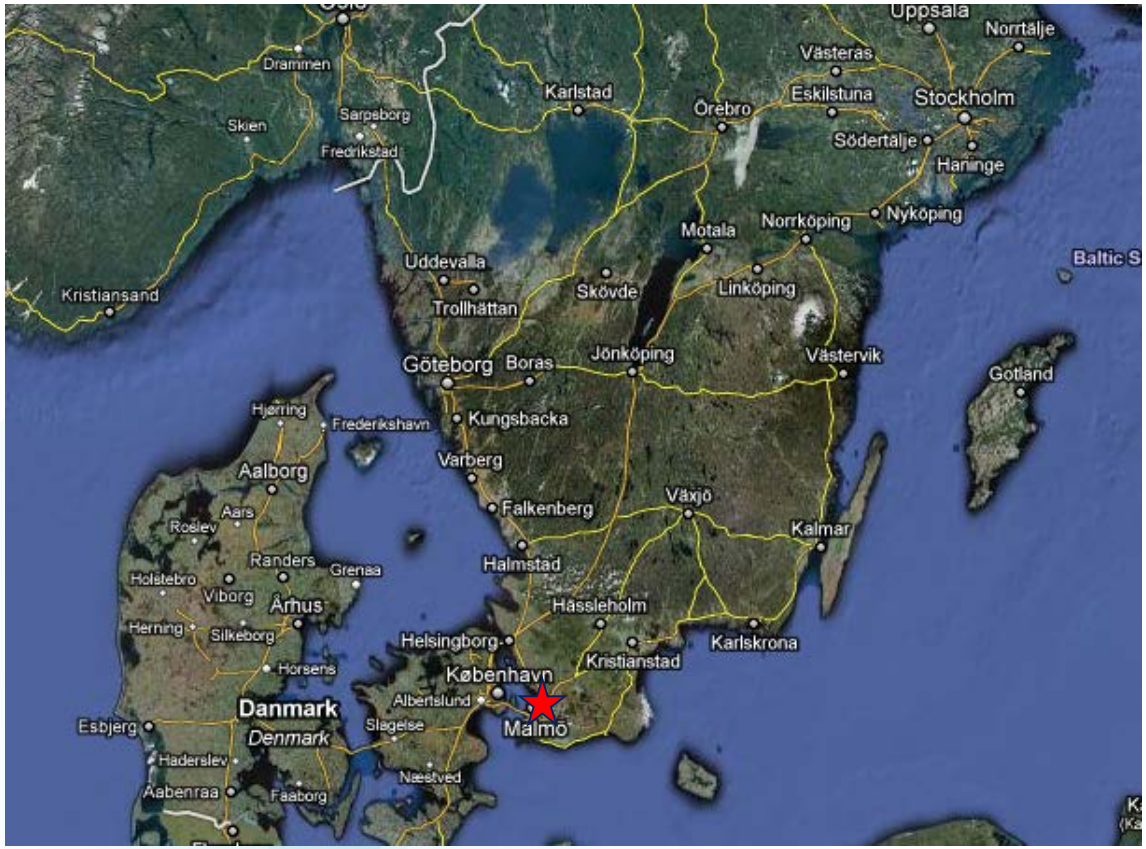
Euro Diagnostica
iLite® Cell-based Reporter Gene Assays

February 2017

Euro Diagnostica

Malmö, Sweden



Euro Diagnostica – in brief

- Founded in 1992
- Family owned by Mr Frederik Paulsen (1)
- Part of Ferring group of companies
  - Ferring Pharmaceuticals (mid-size pharmaceutical company) (2)
  - Nordic Pharma Group (3)
  - PolyPeptide Group (supplier of peptides to pharmaceutical industry) (4)
  - Euro Diagnostica AB
- About 120 employees in Euro Diagnostica worldwide
- Head office in Malmo, Sweden; *iLite*® R&D in Paris, France
- Traditionally, focus has been on auto-immune clinical diagnostics
- Increased focus on assays and services to pharmaceutical industry

1) https://en.wikipedia.org/wiki/Frederik_Paulsen_Jr
2) http://ferring.com/en/home/
3) http://www.nordicpharmagroup.com/
4) http://www.polypeptide.com/

Business Units

Diagnostic Solutions

ELISA and RIA kits
Custom manufacturing, OEM

Euro Diagnostica branded diagnostic IVD kits:
We provide a portfolio of ELISA and RIA kits for various autoimmune diseases and treatment monitoring.

Custom manufacturing:

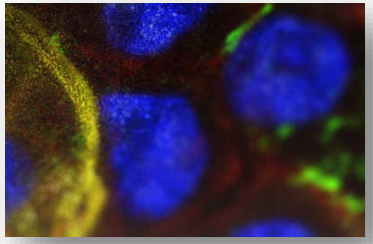
- Top quality plate coating services.
- Antigen production
- Product & production development services.

OEM:

- Collaboration with several market leaders.

Biopharma Solutions

Cell-based assays and development services

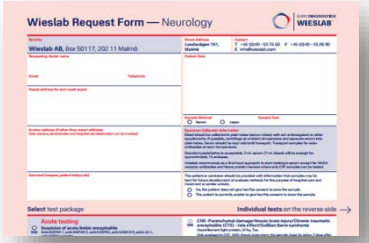


Our cell-based ***iLite®*** assays:

- Provide tools for monitoring and development of biological drugs.
- Are used in customized assays for optimal drug development.

Laboratory Services

Diagnostic & bioanalytical services



Wieslab offers:

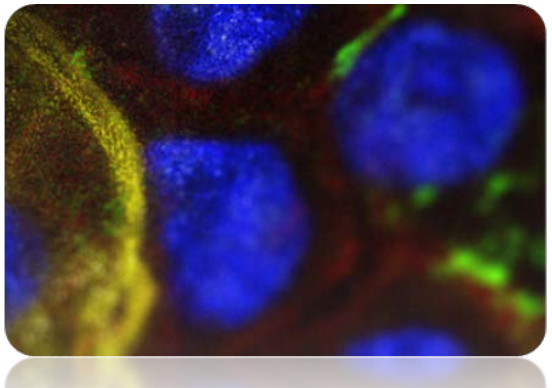
- Clinical testing within diagnosis, prognosis and monitoring.
- Bioanalytical services for non clinical and clinical studies (CRO).
- GLP and ISO 17025 certified environment.

Cell-based assays

Euro Diagnostica merged with Biomonitor in July 2014

- Biomonitor was founded in Copenhagen in 2003. Biomonitor's unique *iLite*® cell-based reporter gene technology developed by Dr. Michael Tovey.
- *iLite*® is a unique reporter gene platform with the ability to measure both drug activity and NABs in biological drugs across a wide range of targets.
- *iLite*® reporter gene cell lines developed for >10 of the world's 20 biggest pharmaceutical companies
- *iLite*® assay ready cells currently used by >50 customers worldwide, mainly US and Europe

- Have been used in a number of FDA and EMA submissions
- TNFα was first cell line to be developed. TNFα cell line used to set Enbrel WHO standard(1)
- TNFα cell line used extensively in clinical monitoring of patients in USA(2,3)



1) "Report on a Collaborative Study for Proposed 1st International Standard for TNF receptor II Fc fusion protein (Etanercept)", WHO, 2015.
2) Pavlov et al., "Clinical laboratory application of a reporter-gene assay for measurement of functional activity and neutralizing antibody response to infliximab", Clinica Chimica Acta, 453 (2016), pp 147-153.
3) Lazar-Molnar et al., "Immunogenicity Assessment of Tumor Necrosis Factor Antagonists in the Clinical Laboratory", Clinical Chemistry, 62:9 (2016), pp. 1186-1198.

iLite® Cell-Based Assays

Off-the-shelf Products

Product Name	Catalogue no.
iLite® TNF-alpha Assay Ready Cells	BM3044
iLite® Type I IFN Assay Ready Cells	BM3049
iLite® Insulin Assay Ready Cells	BM3060
iLite® VEGF Assay Ready Cells	BM4020
iLite® IL-23 Assay Ready Cells	BM4023
iLite® IL-12 Assay Ready Cells	BM4012
iLite® GM-CSF Assay Ready Cells	BM4050
iLite® TLR4 Assay Ready Cells	BM4024
iLite® ADCC Effector (V) Assay Ready Cells	BM4001
iLite® ADCC CD20 (+) Assay Ready Cells	BM4010
iLite® ADCC CD20 (-) Assay Ready Cells	BM4015
iLite® anti-CD20 ADCC Activity Set	BM4070

New *iLite*® Products - Coming Soon!	Catalogue no.
iLite® ADCC HER2 (+) Assay Ready Cells	BM4011
iLite® ADCC HER2 (-) Assay Ready Cells	BM4016
iLite® ADCC EGFR (+) Assay Ready Cells	BM4035
iLite® ADCC EGFR (-) Assay Ready Cells	BM4036
iLite® ADCC Effector (F) Assay Ready Cells	BM4040
iLite® IL-2 Assay Ready Cells	BM4002
iLite® IL-6 Assay Ready Cells	BM4060
iLite® FGF-21 Assay Ready Cells	BM3071

iLite® Cell-Based Reporter Gene Assays
Overview and applications

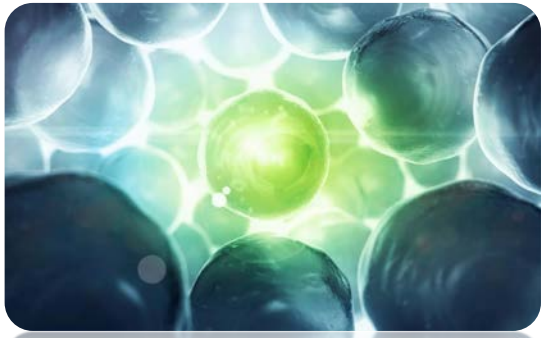
iLite® Cell-Based Assays

Offering & Profile

Euro Diagnostica offers the *iLite* reporter gene technology, in the form of assay ready frozen cells (off-the-shelf products) and as custom cell line development.

Our *iLite* platform is based on reporter gene technology and used in custom made cell-lines for assessment of drug potency and for detection of neutralizing antibodies (NAbs).

Through its specificity for the drug target and a rapid test format, *iLite* cell-lines are valuable during the whole drug development continuum and facilitate the development of new drugs.

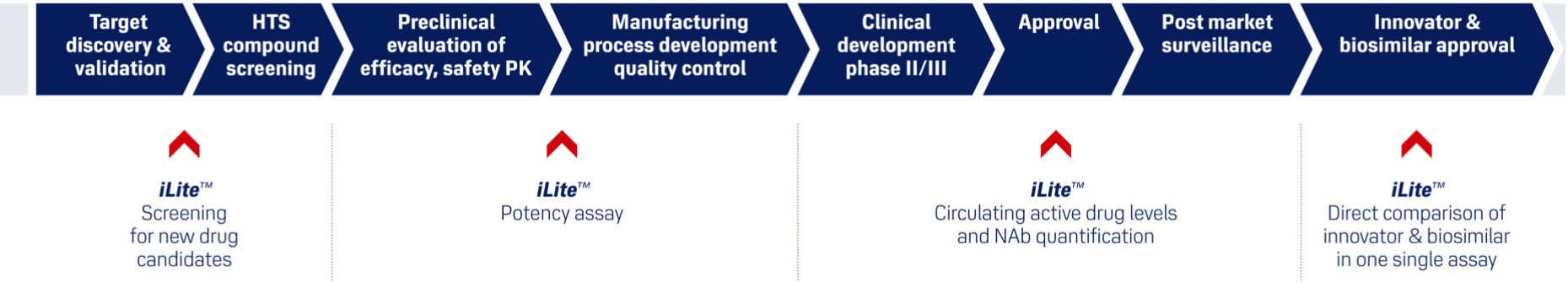


iLite® Cell-Based Assays

Applications

Examples of use of available *iLite* cell lines:

- Functional assessment of a drug (e.g. **potency** determination during manufacturing)
- **Screening** for a drug candidate, with a specific functional target
- Comparison between an innovator drug and a **biosimilar**
- Determination of neutralizing antibodies in **immunogenicity** assessment



Potency assessment

The FDA and EMA recommend bioassays for potency assessment during development / manufacture of biologicals:

"Ideally, the potency assay will represent the product's mechanism of action (i.e., relevant therapeutic activity or intended biological effect)" (1)

"Bioassays can include in vivo animal studies, in vitro organ, tissue or cell culture systems, or any combination of these. You may use in vitro or in vivo assays; however, we encourage the responsible limitation of animal use whenever possible" (1)

"For antibodies for which the clinical activity is only dependent on binding/neutralising properties, a potency assay that measures binding to the target (i.e. binding assay) may be deemed acceptable, if appropriately justified. Where effector functions are relevant for clinical activity, a cell-based bioassay or another assay that takes effector functions into account should be performed" (2)

References:
1. Guidance for Industry: Potency for cellular and gene therapy products, issued by FDA, Jan 2011
2. Development, production, characterisation and specifications for monoclonal antibodies and related products, Revision 1, EMA, Aug 2016

Immunogenicity assessment

Both FDA and EMA recommend bioassays for immunogenicity assessment during development of biologicals:

"Generally FDA considers that bioassays are more reflective (than competitive ligand-binding assays) of the in vivo situation and are recommended" (1)

"FDA recommends that neutralization assays use a cell-based bioassay format depending on the therapeutic protein product's mechanism of action because, frequently, cell-based bioassays more closely reflect the in vivo situation and therefore provide more relevant information than ligand-binding assays." (2)

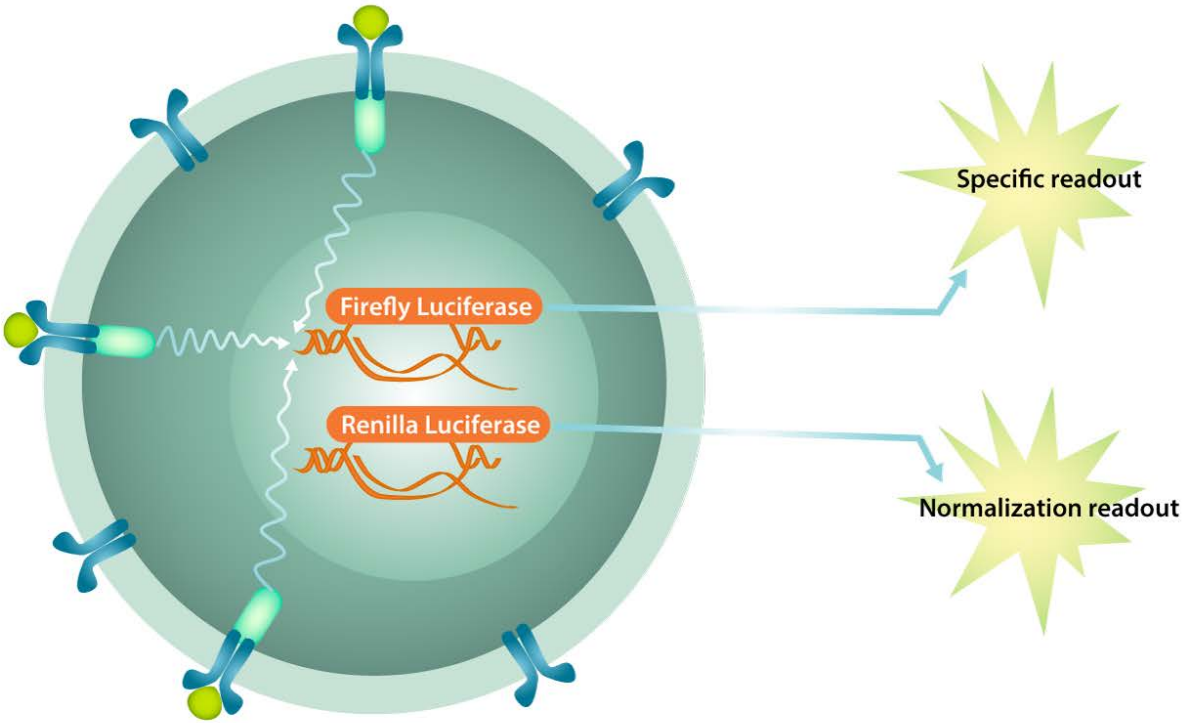
"For most biological products, the most appropriate neutralizing antibody assay is a bioassay which measures the neutralization of the bioactivity" (3)

References:
1. Guidance for Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins, issued by FDA, Dec 2009.
2. Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products, DRAFT GUIDANCE, Apr 2016
3. EMA Guidance on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use, issued by EMA, 2012.

iLite® Cell-Based Reporter Gene Assays
Technology

iLite® Cell-Based Assays

Reporter gene technology



Limitations of Conventional Cell Based Assays* … and the *iLite*® Solution

The *iLite* technology is based on a reporter gene assay format, designed to overcome limitations of conventional cell based assays.

Limitation of Conventional Assays	iLite™ Solution
Subject to variation due to changes associated with continuous cultivation of cells in vitro	Available as Assay Ready Frozen cells; **no cell culturing** needed
Low degree of selectivity	**Highly specific**, receptor selective signal
Subject to non-specific interference including serum matrix effects	**Normalization** of cell counts and serum matrix effects by a second reporter gene
Take several days to complete	Results **within one workday**
Based on complex biological endpoints that are difficult to quantify with precision	Endpoint is light emission easily quantified with a standard luminometer

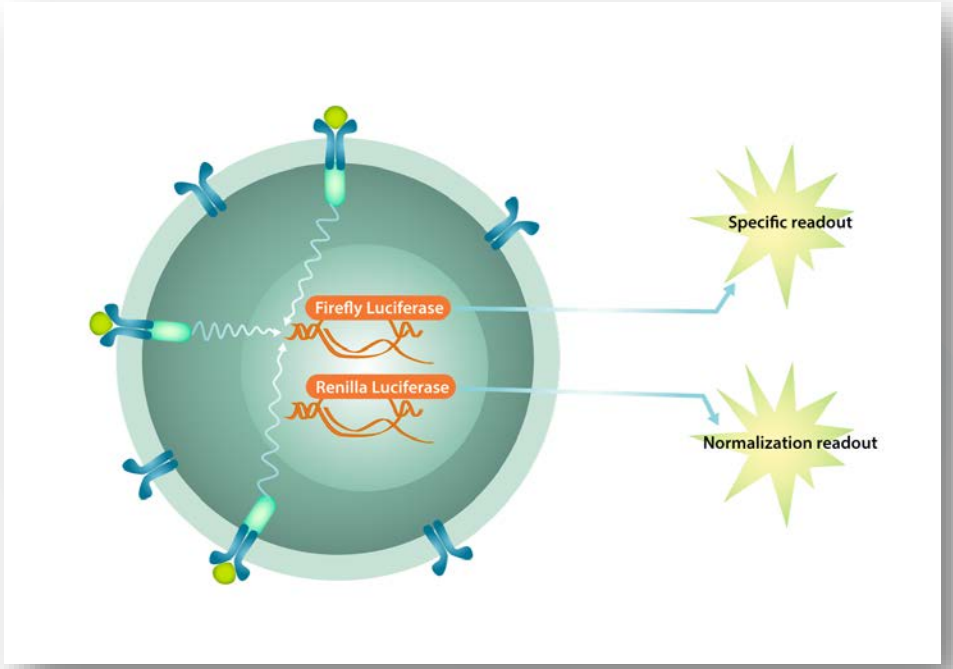
**Conventional cell-based assays includes proliferation and stimulation assays etc.*

iLite® Cell-Based Assays

Technology

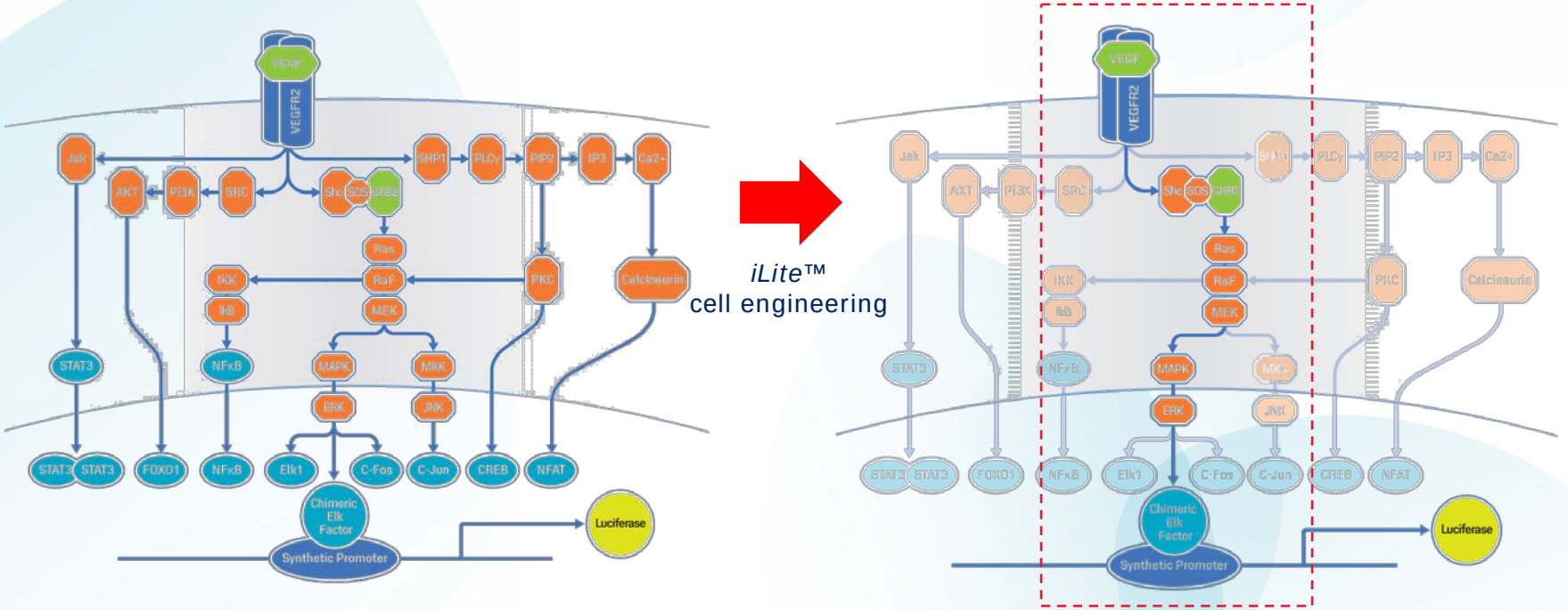
The special features of the patented *iLite*™ technology are:

- Highly **specific** reporter gene cell lines
- Very **sensitive** cell line responses (>10 fold inductions)
- Assay Ready Cells – **ready-to-use** from the freezer, without culturing of cells
- Assays **within a workday** (typically 4-7 hour assays)
- **Normalization** gene, which eliminates unwanted matrix effects



iLite® Cell-Based Assays

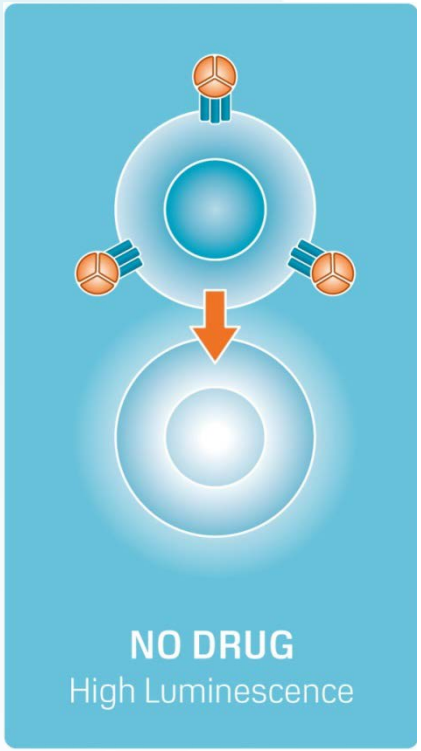
Pathway Specific

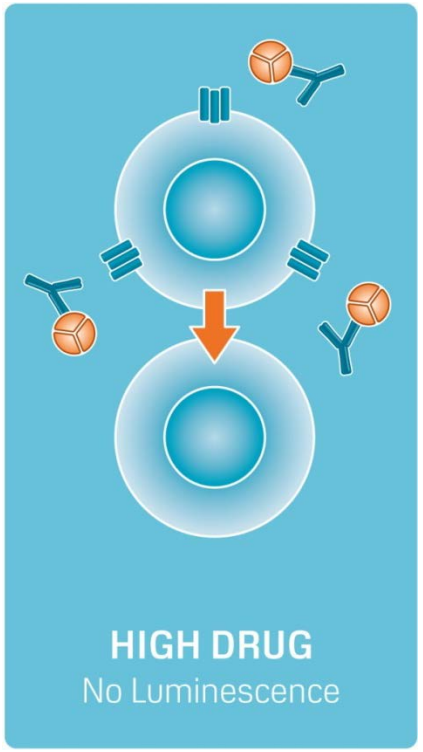


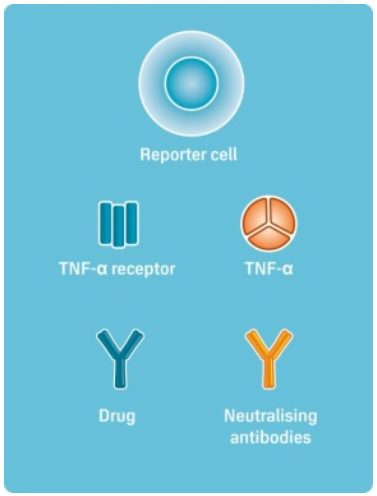
iLite™ VEGF Assay Ready Cells

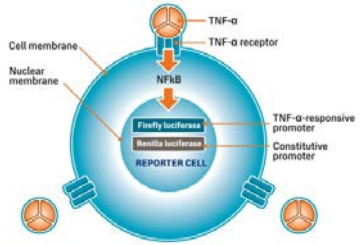
Detection of Drug Activity

Exemplified by TNF-alpha Assay Ready Cells



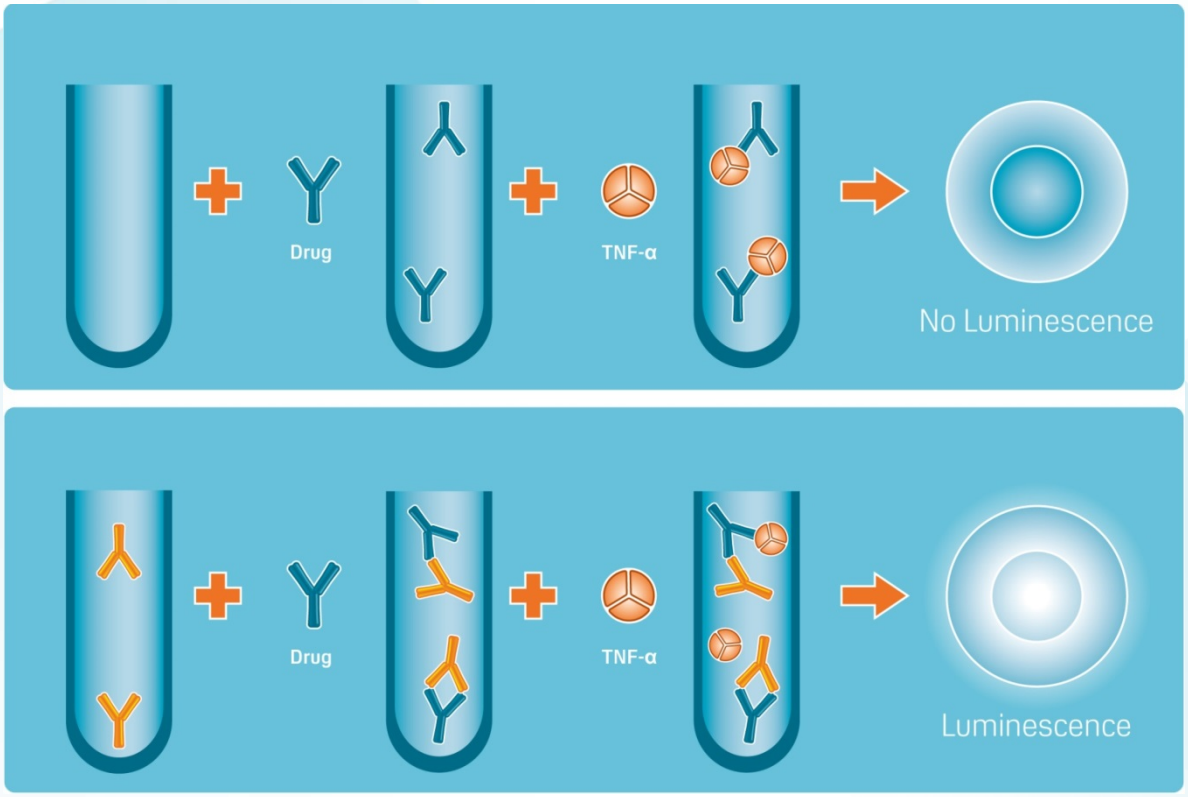


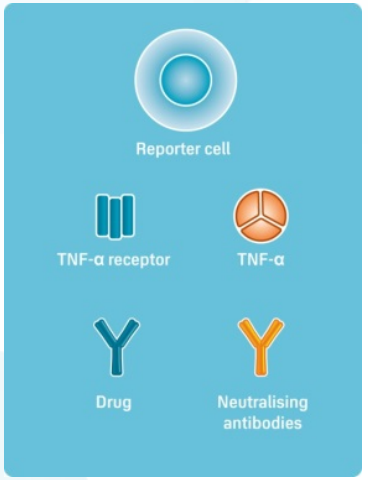


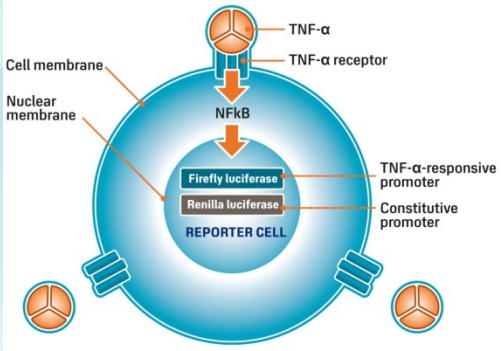


Detection of Drug activity or NAbs in Serum

Exemplified by TNF-alpha Assay Ready Cells



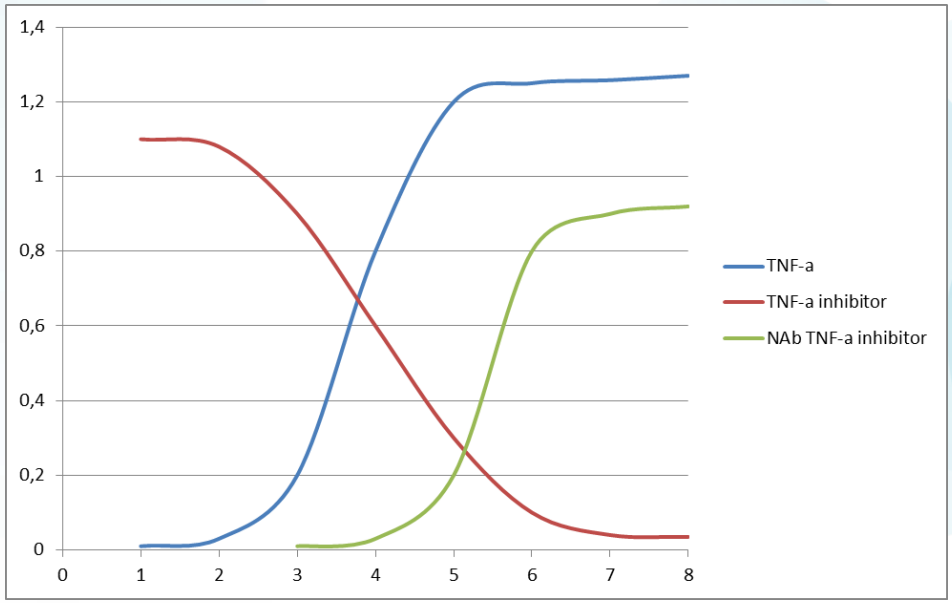




Assay Read-Out

Response curves as exemplified by TNF-α Assay Ready Cells

- Cells treated with:
  - TNF-α
  - TNF-α and TNF-α inhibitor (measure drug activity)
  - TNF-α and TNF-α inhibitor in the presence of NAbs (detection of Nabs)

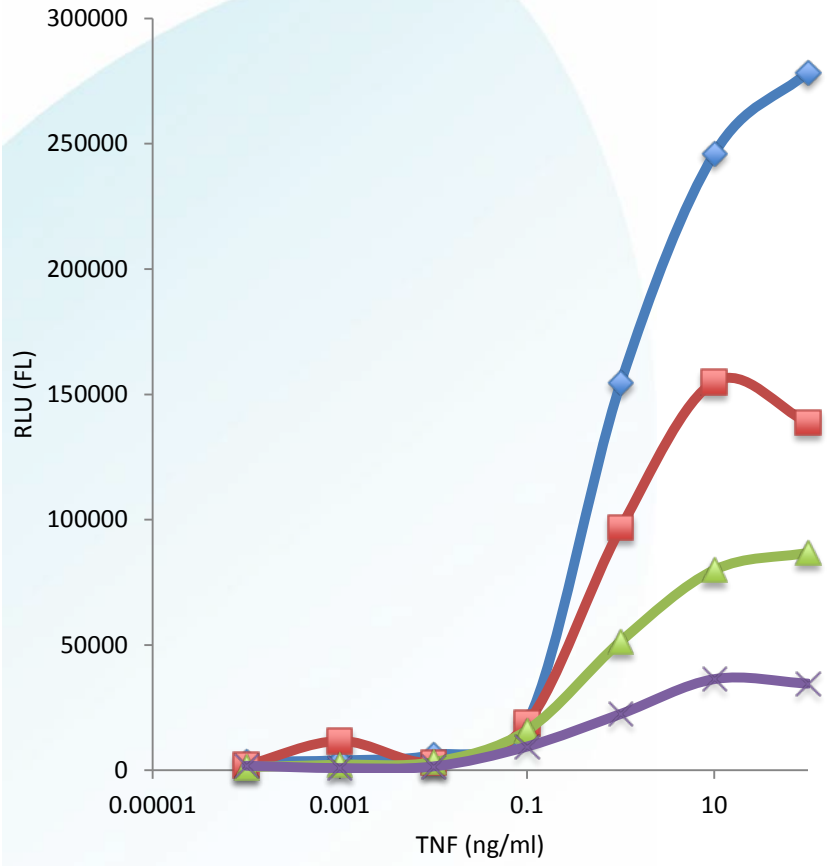


iLite® Cell-Based Reporter Gene Assays
Performance Data

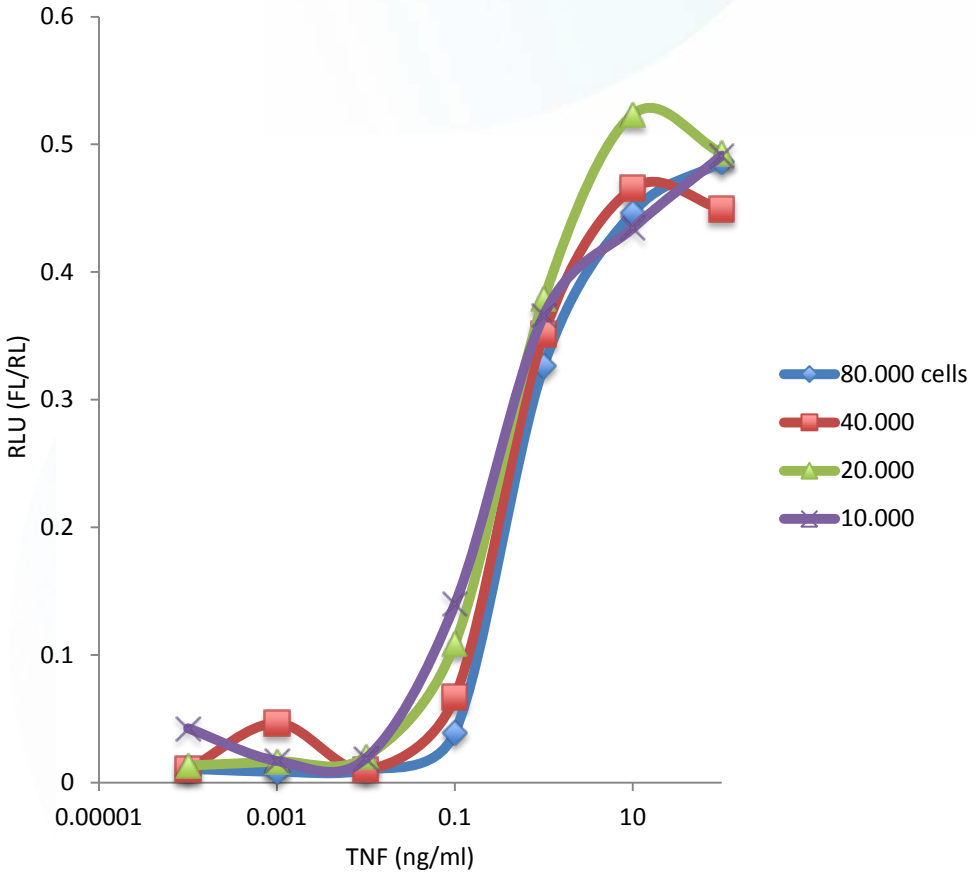
iLite® Normalization

Results are independent of cell number

Without normalization



With normalization

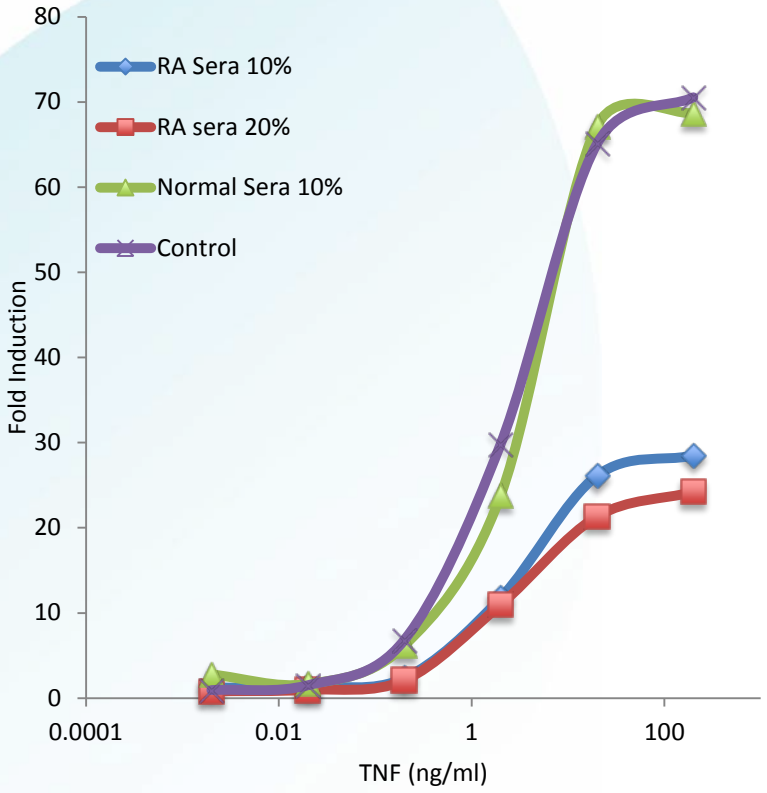


Adapted from Lallemand et al. (2011) Journal of Immunological Methods

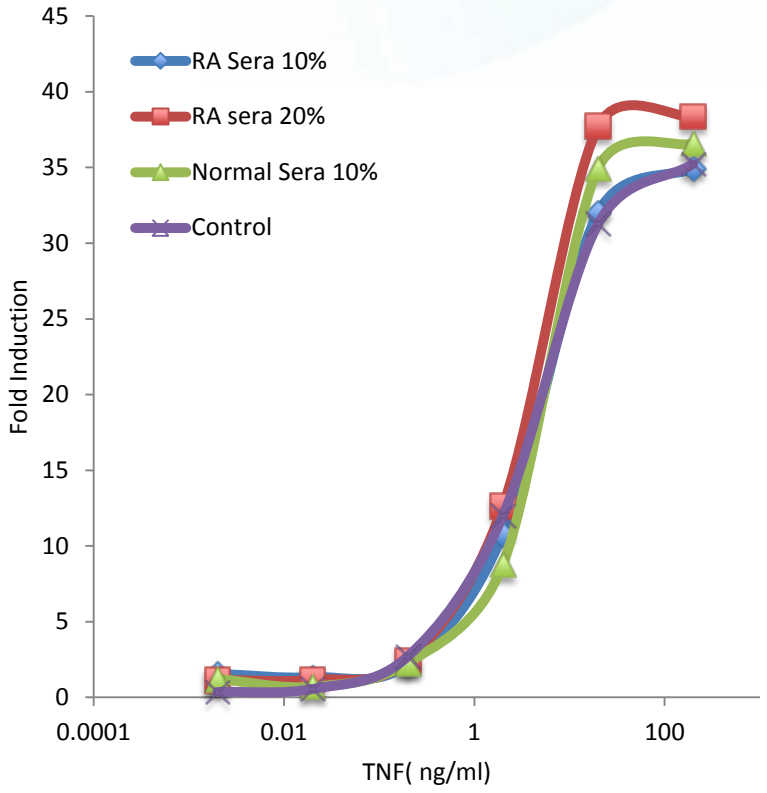
iLite® Normalization

Eliminates Serum Matrix Effects

Without Normalization

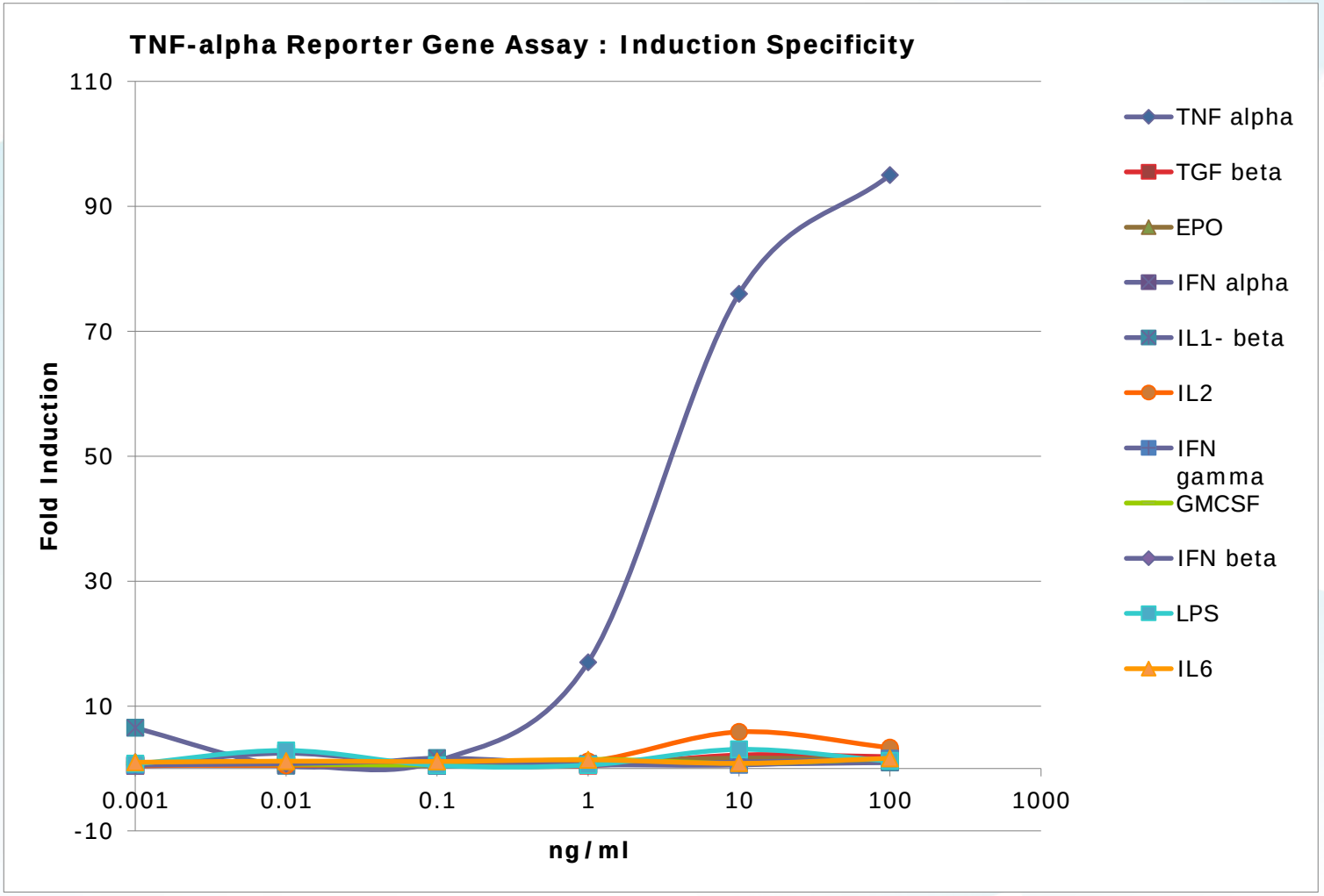


With Normalization



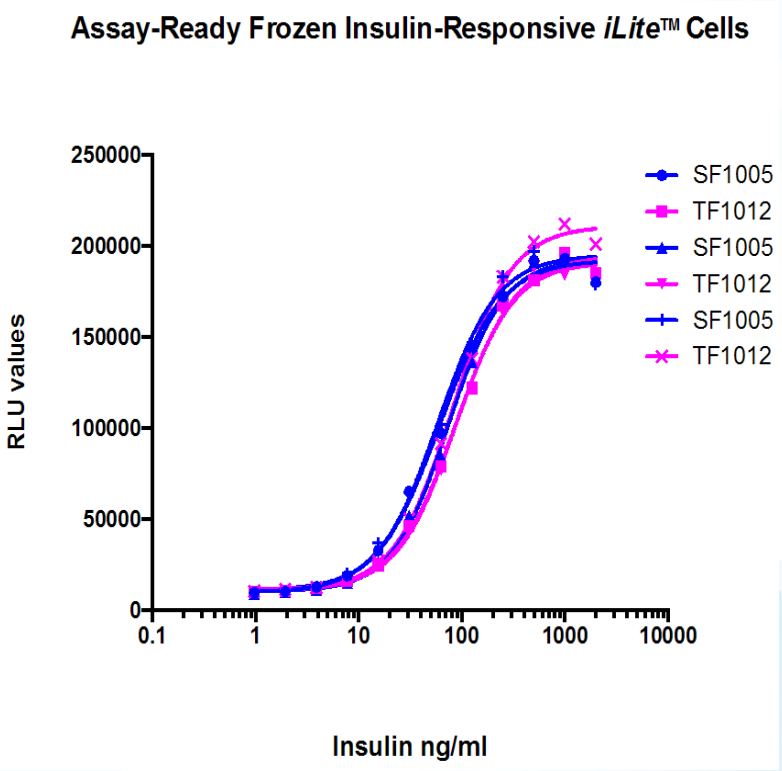
Adapted from Lallemand et al. (2011) Journal of Immunological Methods

Specificity and Sensitivity



Adapted from Lallemand et al. (2011) Journal of Immunological Methods

Batch Reproducibility

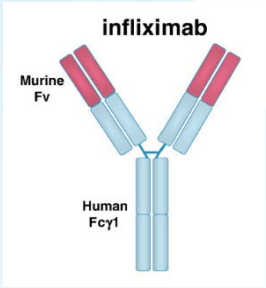


Parameter	SF1005	SF1005	SF1005	TF1012	TF1012	TF1012	CV %
Top	191944	194577	192708	194132	190829	210681	3,79
Bottom	9821	11006	10308	10697	11455	10958	5,38
Log EC50	1,782	1,768	1,868	1,942	1,938	1,911	4,12
Hill slope	1,454	1,52	1,613	1,491	1,55	1,532	3,54
Span	182123	183570	182400	183435	179374	199723	3,95

Structure of TNF blockers

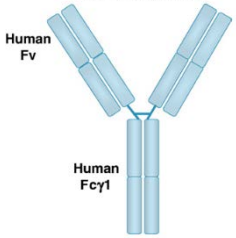
Monoclonal antibodies

Remicade®

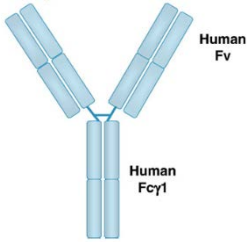


Mix between murine and human IgG sequenes

Humira®



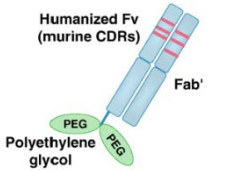
Simponi®



Fully human IgG sequences

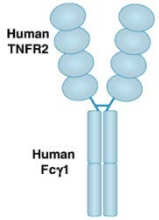
Chimeric proteins

Cimzia®



Antibody binding regions from human / mouse IgG linked to PEG

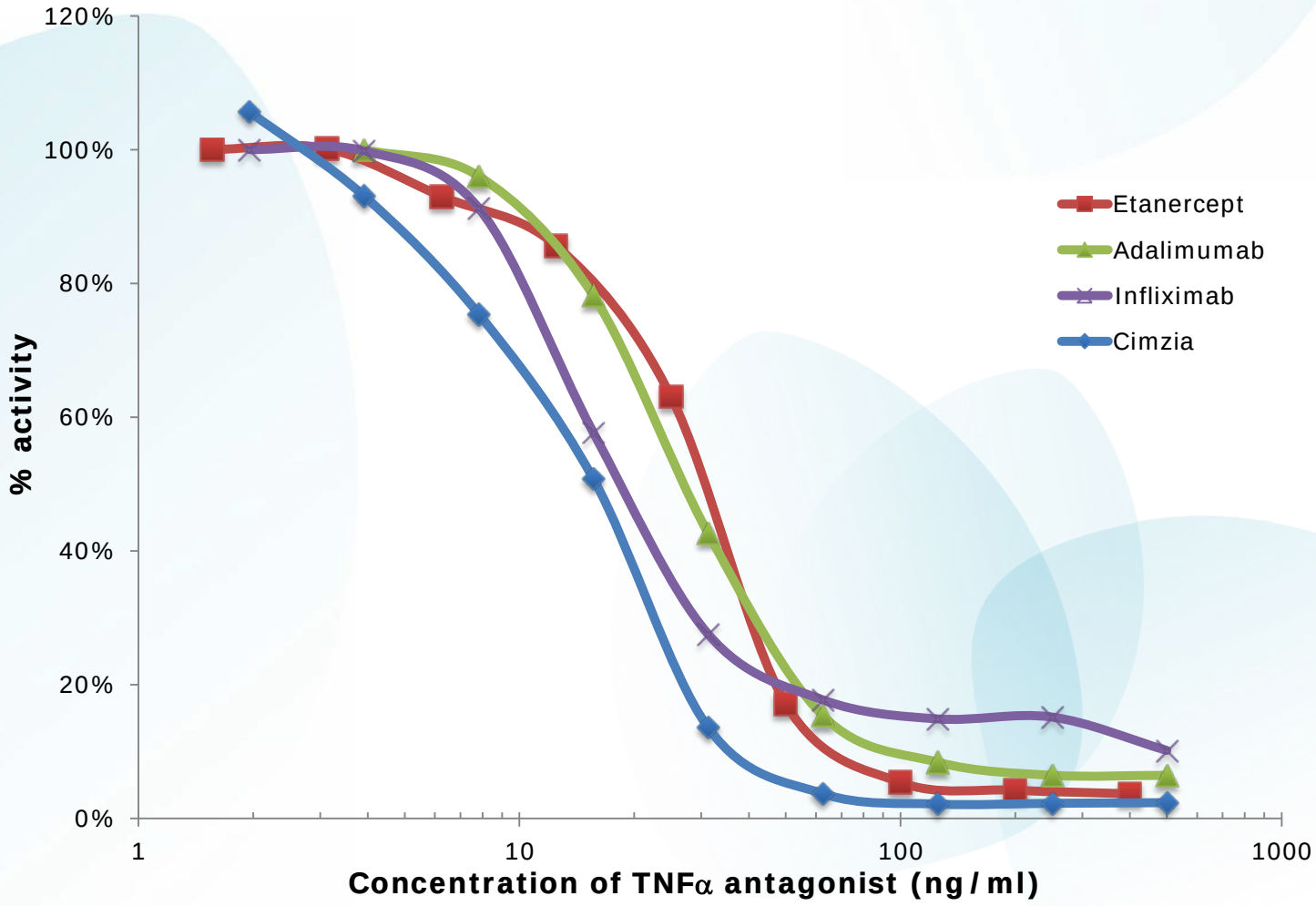
Enbrel ®



Human TNFR2 linked to Fcγ1 from IgG

D. Tracey et al. / Pharmacology & Therapeutics 117 (2008) 244–279

iLite® - A Single Assay to Measure Structurally Diverse Drugs



iLite® Cell-Based Reporter Gene Assays
Practicals

Ease of Use

Sophisticated but Simple to Perform

- **No cell culturing** - *iLite*® Assay Ready Cells do not require culturing, are used directly from the freezer
- All assays are run in **standard 96 well format**
- **As easy as running an ELISA** but no washing steps
- All assays can be performed within **one workday**

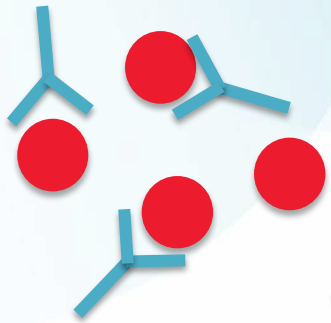
Simple Assay Procedures

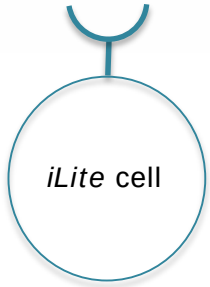
Pre-incubation
- Dilute samples (and mix with target)
- Incubate (37°C, 5% CO_2) – **30 min**

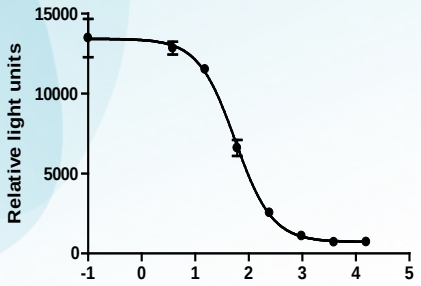
Addition of cells
- Thaw, dilute and add cells
- Incubate (37°C, 5% CO_2) – **3-7h**

Develop light reaction
- Add substrate
- Incubate (RT) – **10 min**

Read plate
- Read plate in a luminometer







iLite® Cell-Based Reporter Gene Assays
Product details

iLite® Cell-Based Assays

Off-the-shelf Products

Product Name	Catalogue no.
iLite® TNF-alpha Assay Ready Cells	BM3044
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iLite® Insulin Assay Ready Cells	BM3060
iLite® VEGF Assay Ready Cells	BM4020
iLite® IL-23 Assay Ready Cells	BM4023
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iLite® anti-CD20 ADCC Activity Set	BM4070

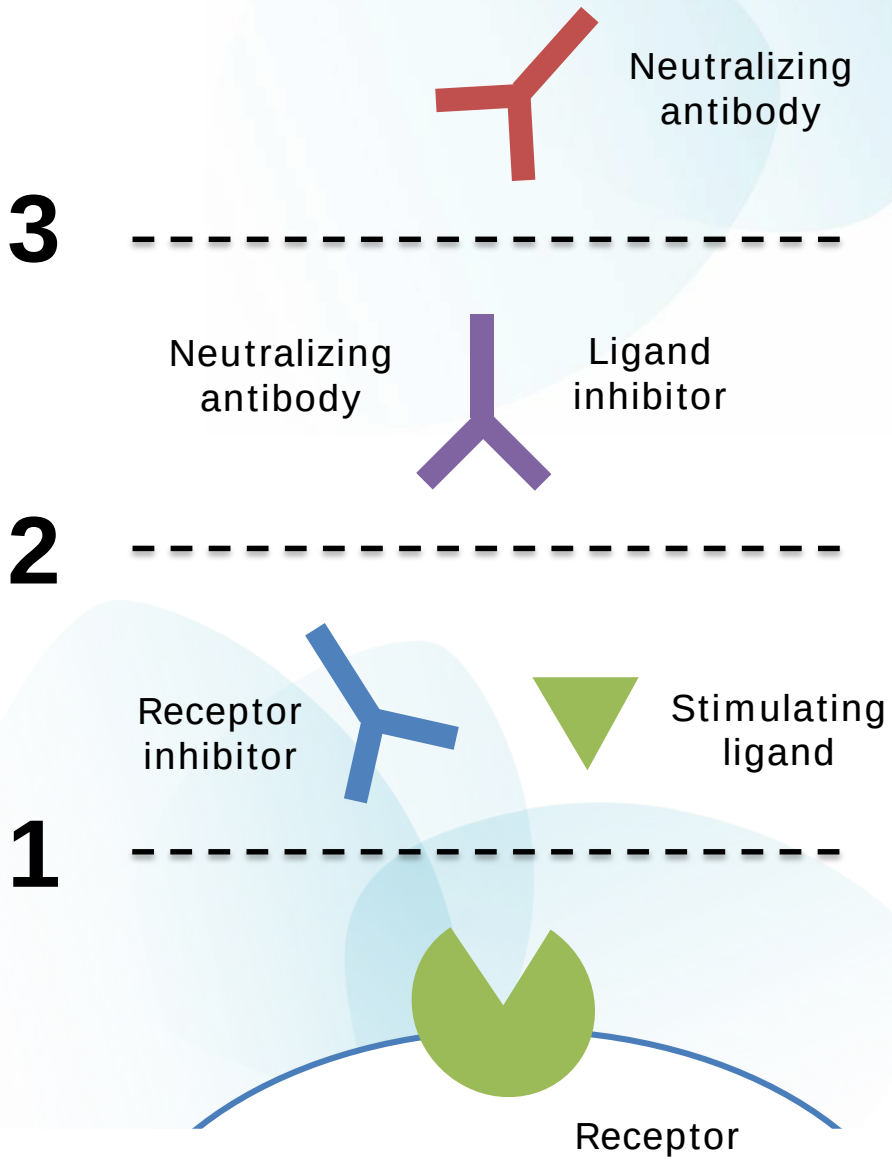
New *iLite*® Products - Coming Soon!	Catalogue no.
iLite® ADCC HER2 (+) Assay Ready Cells	BM4011
iLite® ADCC HER2 (-) Assay Ready Cells	BM4016
iLite® ADCC EGFR (+) Assay Ready Cells	BM4035
iLite® ADCC EGFR (-) Assay Ready Cells	BM4036
iLite® ADCC Effector (F) Assay Ready Cells	BM4040
iLite® IL-2 Assay Ready Cells	BM4002
iLite® IL-6 Assay Ready Cells	BM4060
iLite® FGF-21 Assay Ready Cells	BM3071

iLite® Cell-Based Assays

Off-the-shelf Reagents

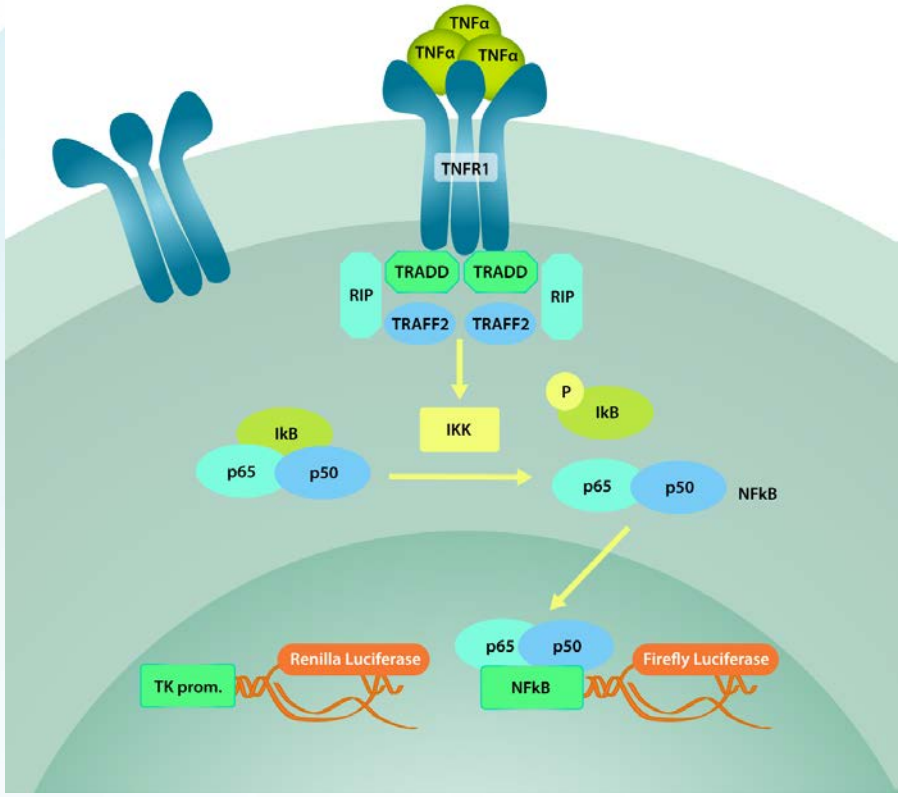
Product Name	Catalogue no.
iLite® TNF-alpha Assay Ready Cells	BM3044
iLite® TNF-alpha (16 ng/mL)	BM3133
iLite® Infliximab NAb positive control	BM3136
iLite® Adalimumab NAb positive control	BM3159
iLite® Etanercept NAb positive control	BM3177
iLite® Diluent A	BM3132
iLite® Diluent B	BM3134
iLite® Diluent C	BM3139
iLite® Reagent BLANK	BM3135
iLite® Type I IFN Assay Ready Cells	BM3049
iLite® IFN beta 1a (950 IU/mL)	BM3249
iLite® IFN beta 1a NAb positive control	BM3251
iLite® Diluent D	BM3250

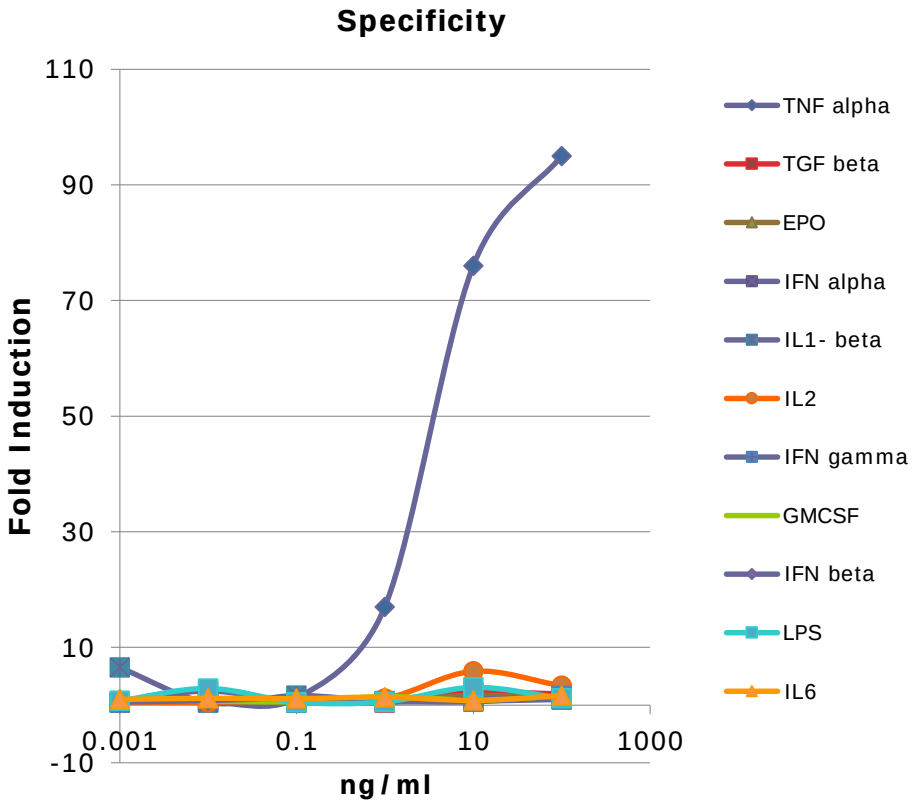
iLite® Assay Ready Cells	Measurable event
TNF-α	1, 2, 3
Type I IFN	1, 2
Insulin	1, 2
IL-23	1, 2, 3
IL-12	1, 2, 3
VEGF	1, 2, 3
GM-CSF	1, 2, 3
TLR4	1, 2



iLite® TNF-α Assay Ready Cells

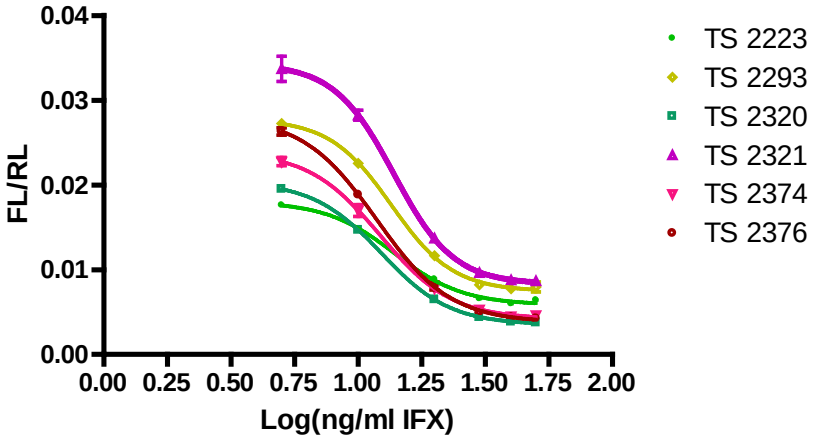
High specificity and sensitivity





iLite™ TNF-α Assay Ready Cells

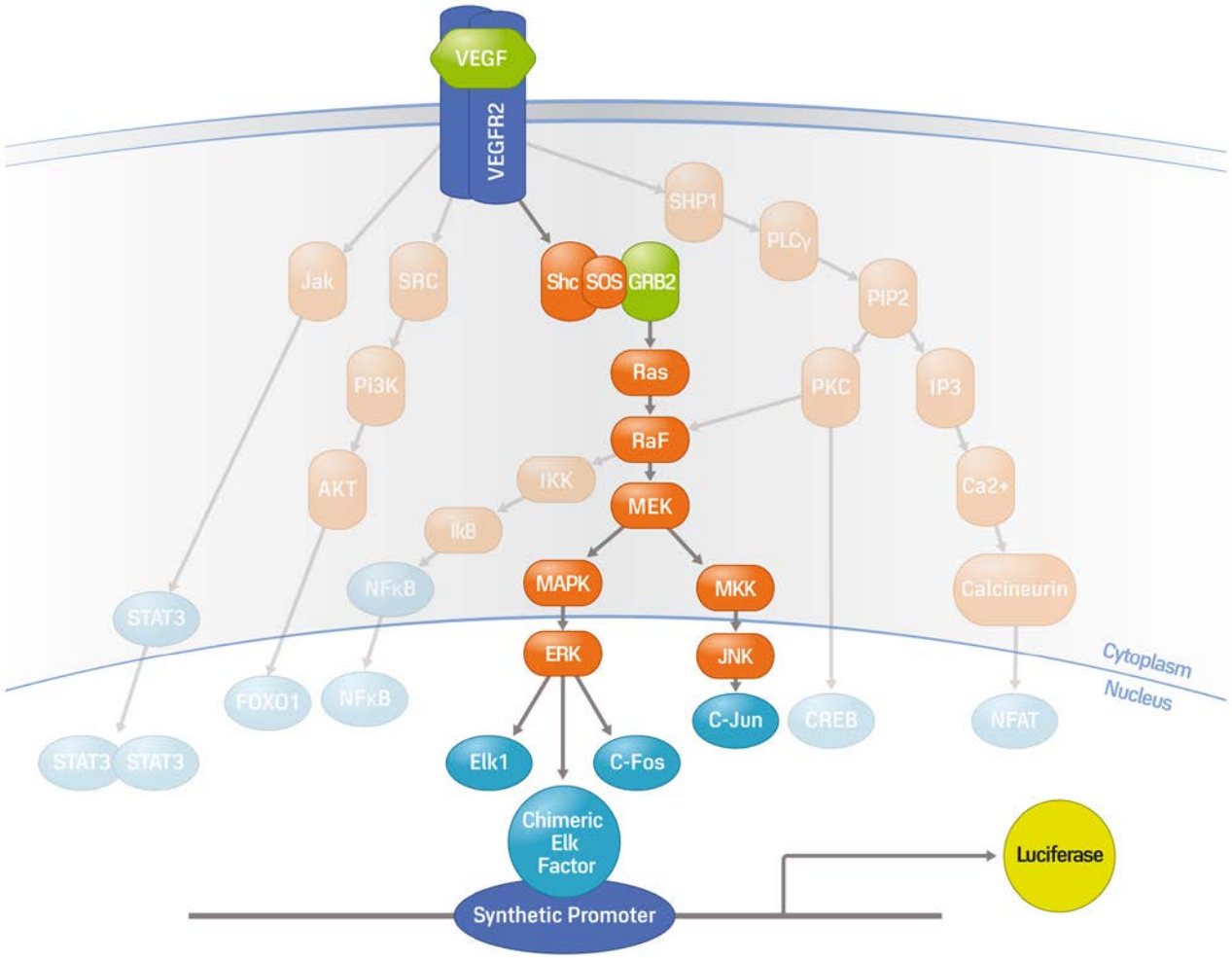
High reproducibility



Parameter	TS 2223	TS 2293	TS 2320	TS 2321	TS 2274	TS 2376	%CV
Positive control (ng/ml IFX)	22,6	23,8	23,4	22,9	24,3	25,1	3,9
Hillslope	-3,221	-3,575	-3,213	-3,685	-3,073	-2,987	8,4
EC50	13,9	13,52	12,35	13,83	12,21	11,83	7,1
Max-Min RLU (span 5 - 50 ng/ml IFX)	15655	20462	17616	23304	18064	20474	14

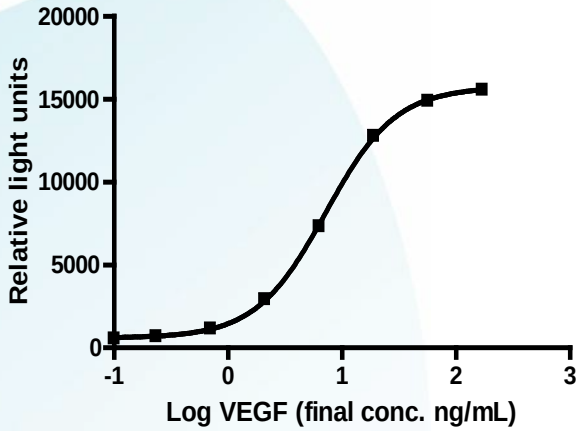
iLite® VEGF Assay Ready Cells

High specificity

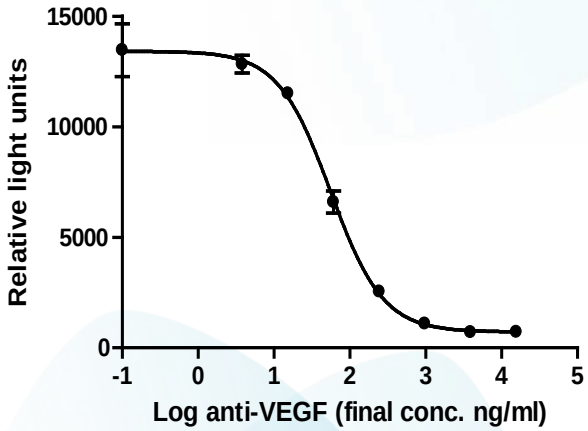


Response curves

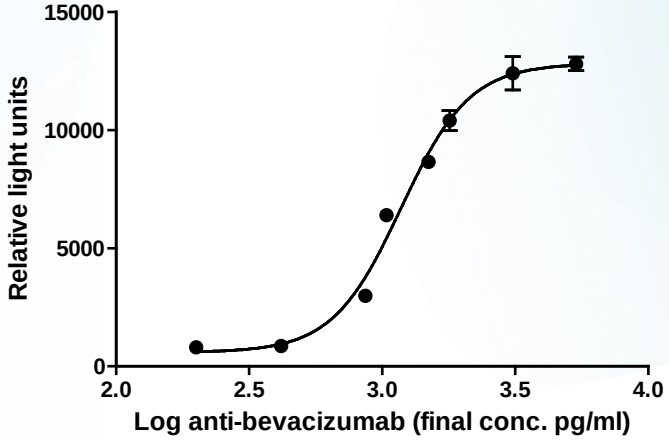
VEGF activity



VEGF inhibitor activity

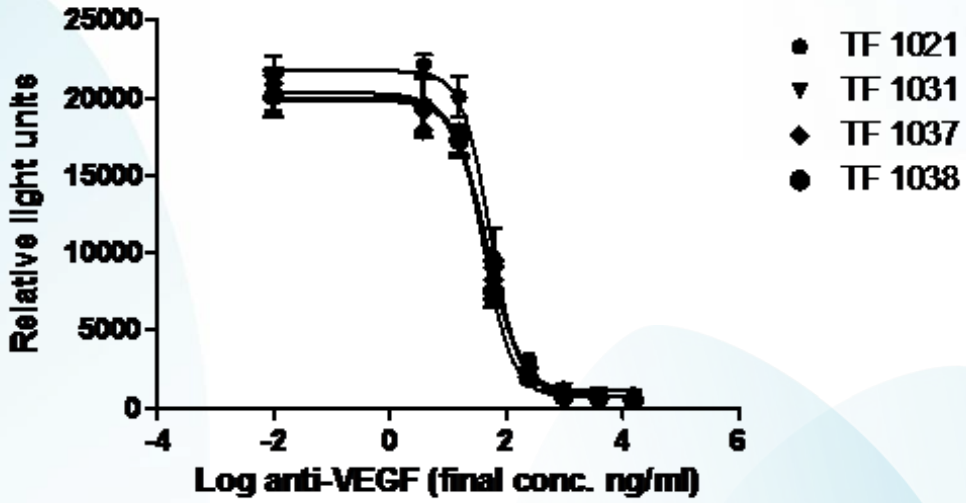


NAbs towards VEGF inhibitor



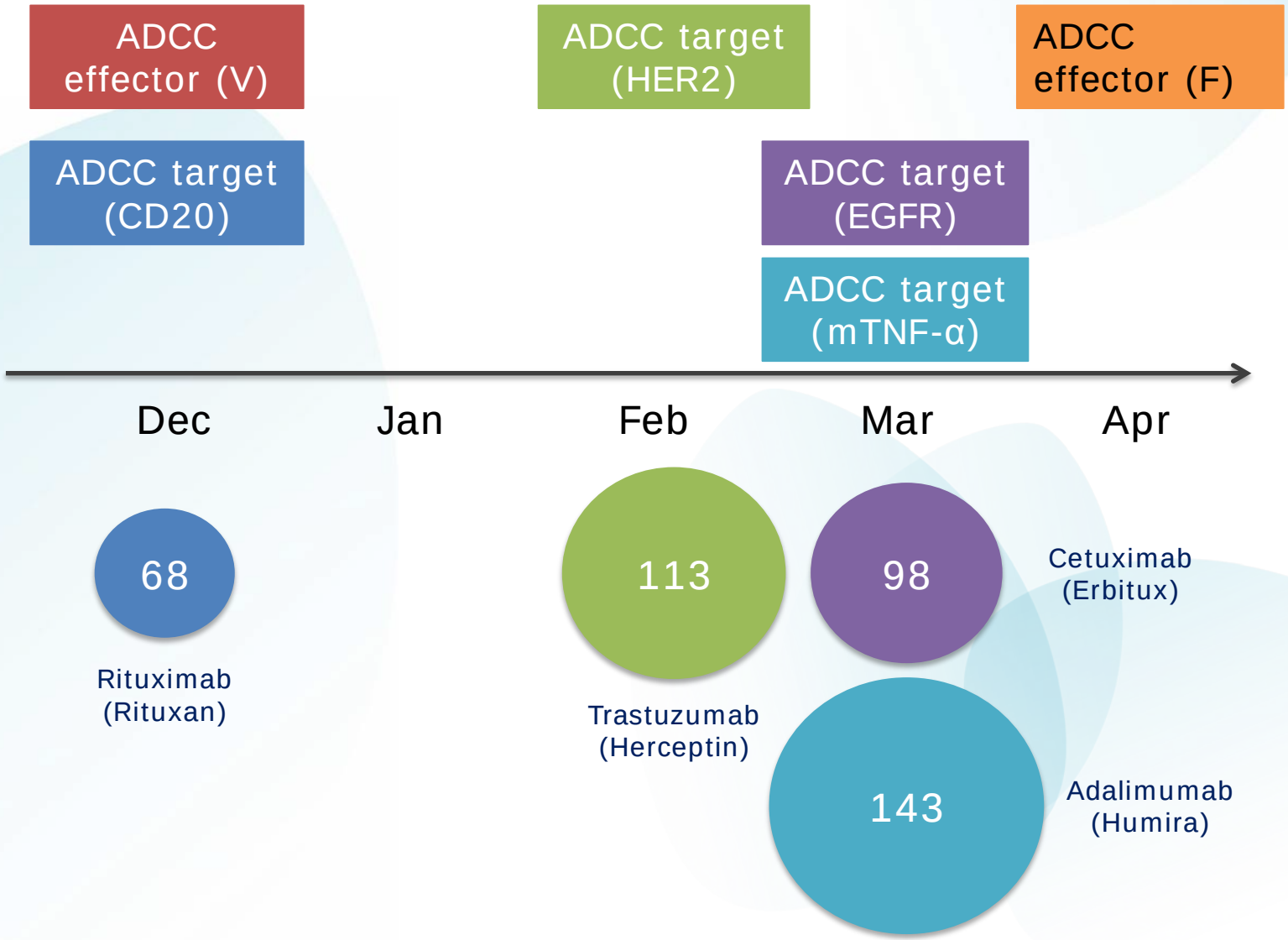
Batch reproducibility

Bevacizumab activity

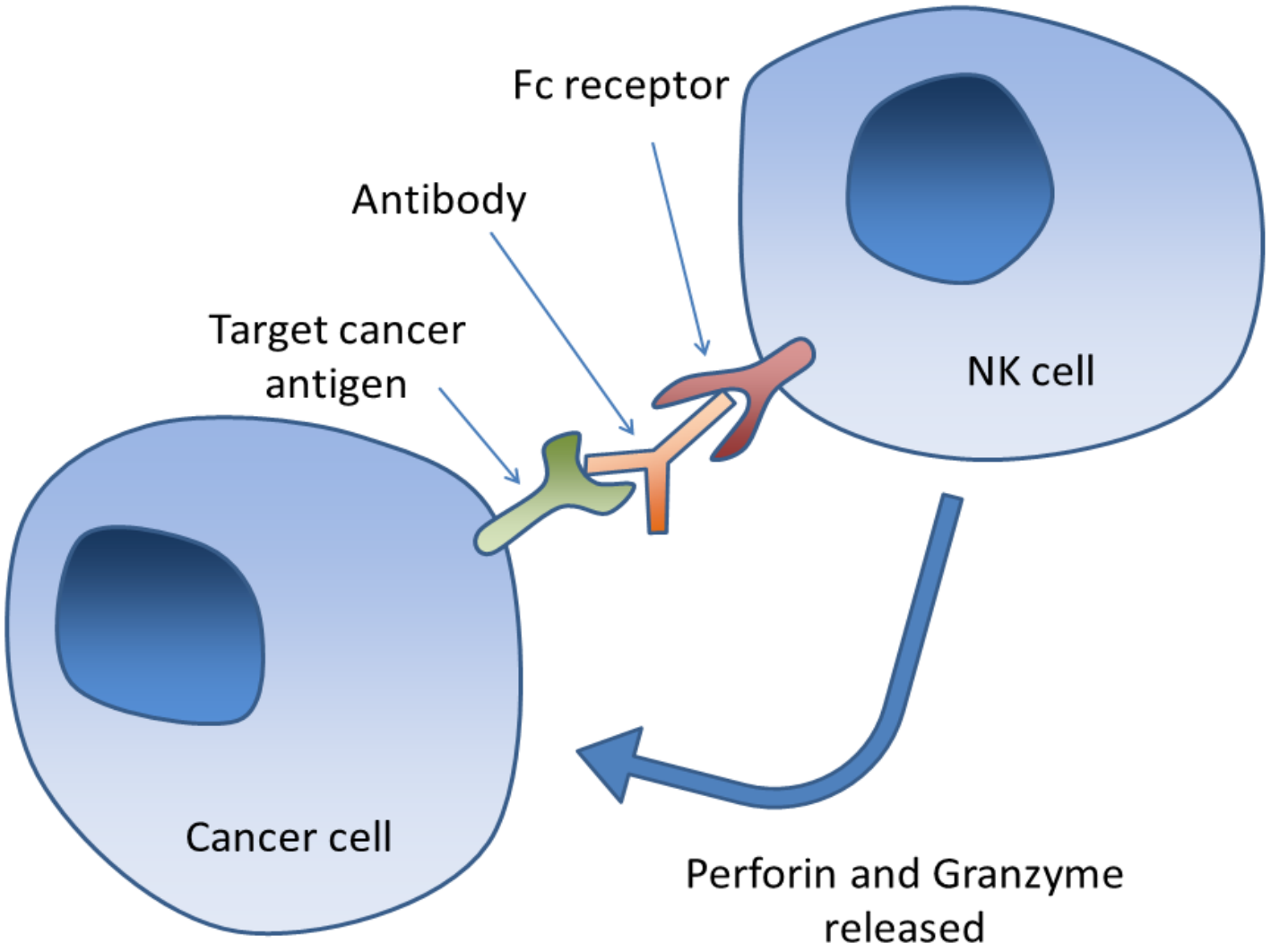


Parameter	TF 1021	TF 1031	TF 1037	TF 1038	CV%
Hill Slope	-1.799	-1.382	-1.542	-1.729	12
IC50	52.22	45.52	48.29	41.53	10
Assay Span (Max-Min RLU)	20092	20201	20313.5	19646	1.5

New Product Line - *iLite*® ADCC Activity Assays



Antibody Dependent Cell Cytotoxicity



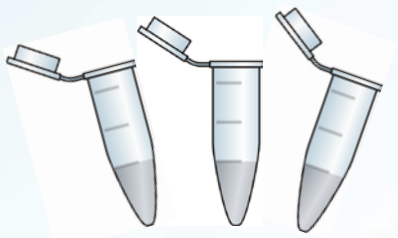
iLite® ADCC Product Line
General Product Set-Up

iLite® ADCC Effector Assay Ready Cells

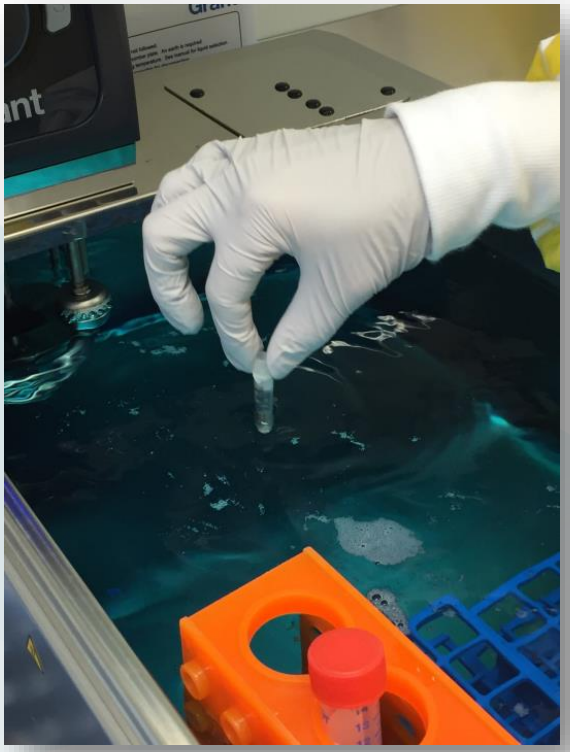
iLite® ADCC Target (+) Assay Ready Cells

iLite® ADCC Target (-) Assay Ready Cells

➢ *iLite*® Target ADCC Activity Set

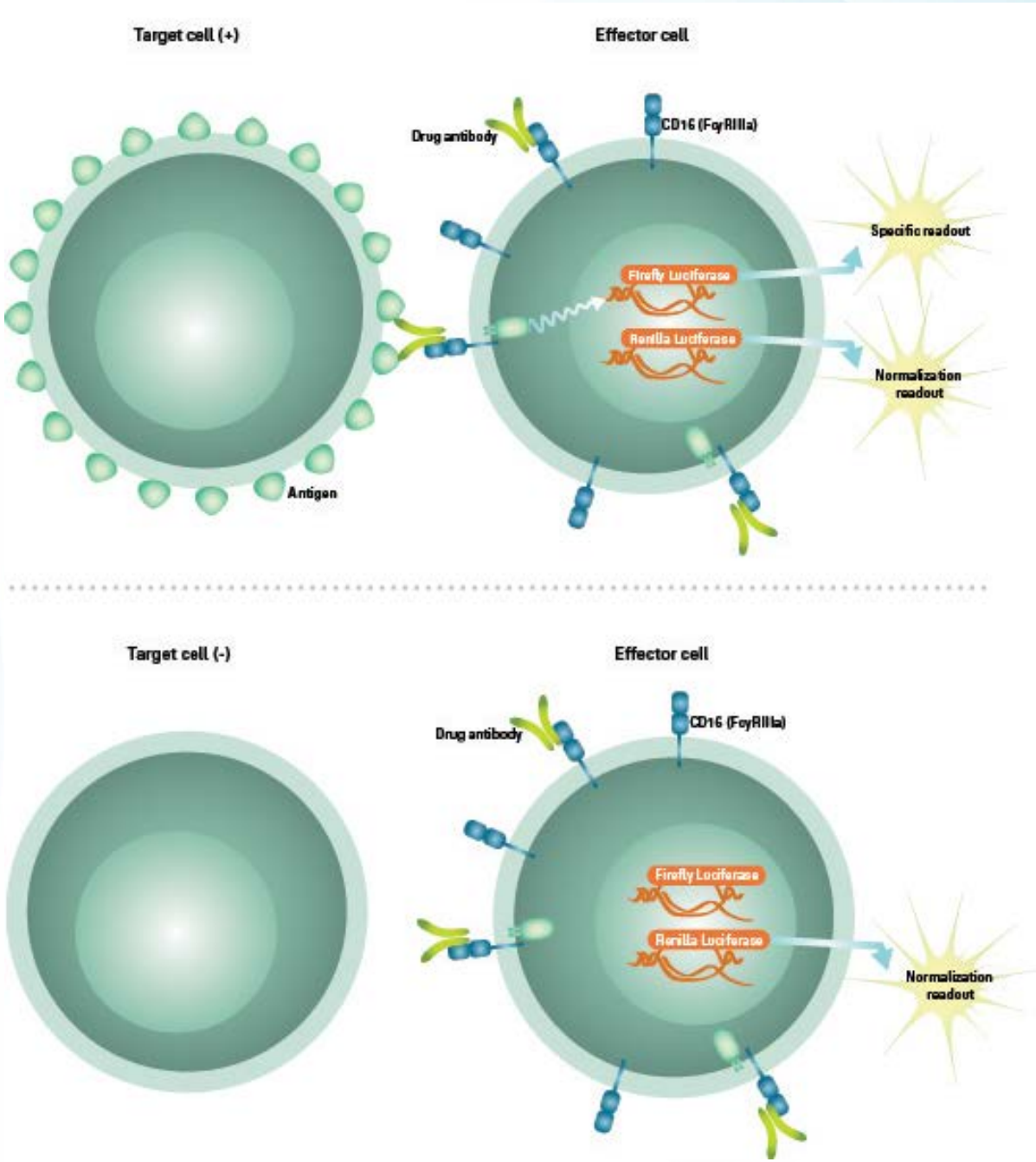


Effector, Target + and Target -



Unique features!

- Target +
- Target –
- Normalization
- Serum tolerance



iLite® ADCC CD20 Assay Ready Cells

Product information

CD20 is a surface protein expressed primarily on B-cells. The first antibody-based therapeutic against cancer, Rituximab, targets CD20. In hematological cancers, B-cells become cancerous and divide uncontrolloably. The drug's mechanism of action is in part to induce ADCC targeting the CD20 expressing tumor cells.

No. of biopharmaceutcals in pipeline: 68

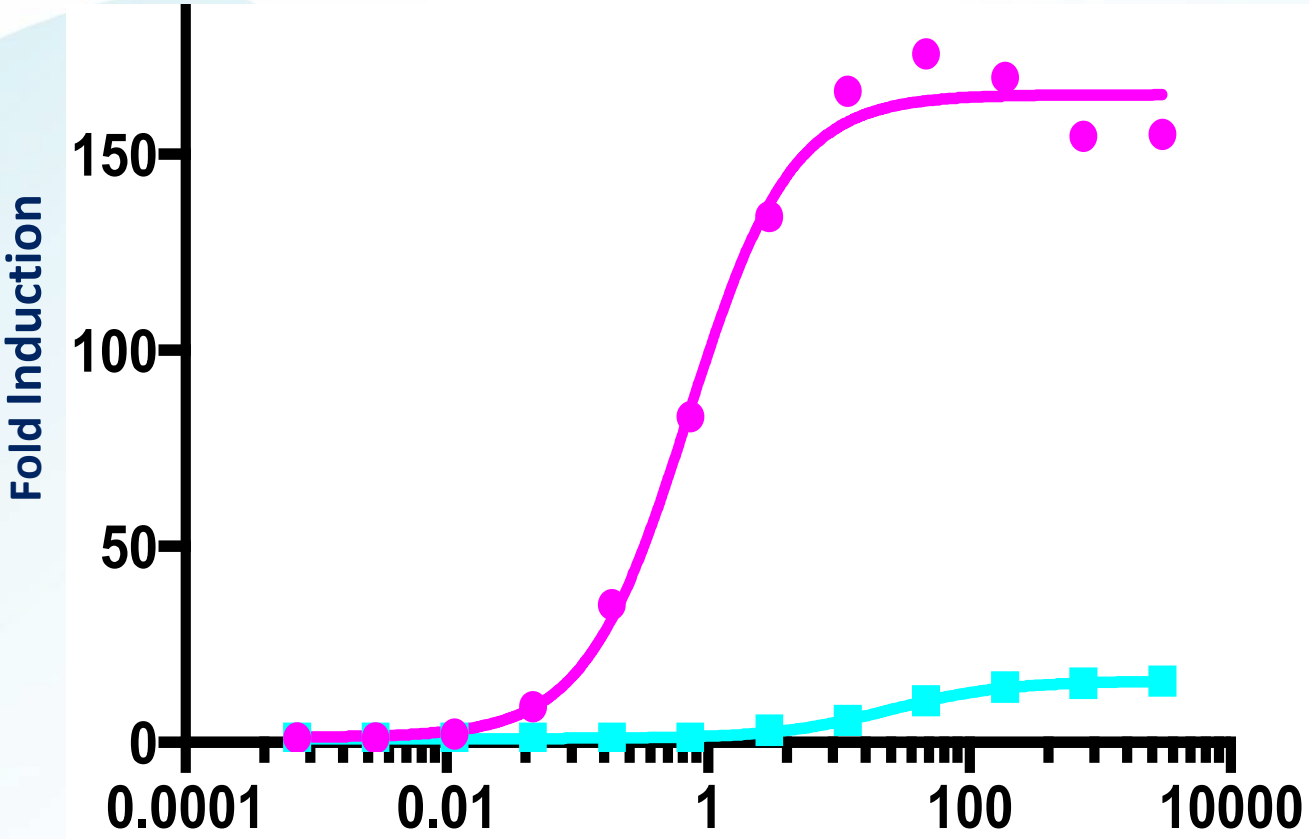
With *iLite* ADCC CD20 Assay Ready Cells, unique on the market, you can measure:

- The ADCC activity of anti-CD20 drugs in the presence of serum (5h)

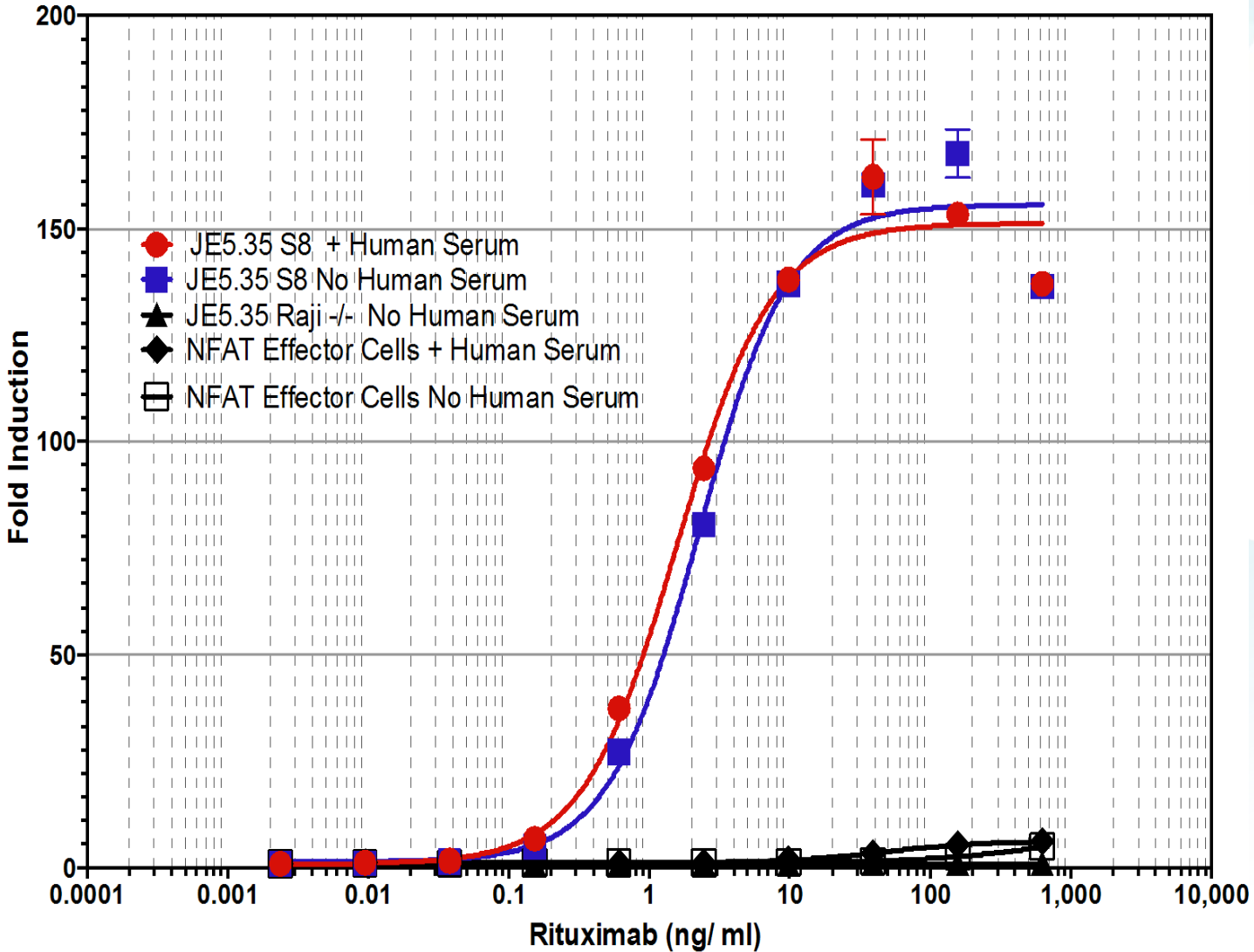
iLite® CD20 ADCC assay performance
Summary

Parameter	*iLite* Assay-Ready Effector Cells	Commercially available Effector Cells
Promoter	NFAT-AP1-NFkB-CEBP	NFAT
E:T Ratio	3:1	6:1
Fold induction	**150 fold**	**10 fold**
Incubation Time	**4 hours**	**6 hours**
EC50	**2 ng/ml**	**120.0 ng/ml**
LLOQ	**1 pg/ml**	**10.0 ng/ml**
Normalization Gene	**+**	**-**
Substrate	Dual-Glo or Bio-Glo	Bio-Glo
Results influenced by loss of effector cells	NO	YES
Results influenced by serum matrix effects	NO	YES
Results influenced by target cell killing of effector cells	NO	YES

iLite® CD20 ADCC assay vs. commercially available assay



iLite® CD20 ADCC assay vs. commercially available assay



iLite® Cell-Based Reporter Gene Assays
Custom Assay Development

iLite® Custom Assay Development

General Work Plan

- Development projects performed by Dr. Tovey's team in Paris
- Projects are split into work packages, executed sequentially and/or in parallel

- Bi-weekly or monthly telecons for updates depending on project phase
- Validations can be performed by Euro Diagnostica's in-house bioanalytical service laboratory

- Project proposal
- Master service agreement

iLite® Custom Assay Development

General Work Plan

Projects typically initiated by a larger pharmaceutical company

Work packages generally include:

- Scientific project design
- Stable transfectant established
- Sub-clone
- Master cell bank
- Working cell bank
- Defined cell replication step
- Stability testing
- Cryo-preservation
- Manufacture of Assay Ready Cells

Project timeline 3-6 months

Thank You!

Euro Diagnostica received the

2013 Frost & Sullivan European Best Practice Award

for Product Differentiation Excellence in Autoimmune Disease Diagnostics

AND

2015 Frost & Sullivan European Best Practice Award

for Customer Value in Theranostic Services