

*Quantification of Neutralizing Antibodies to  
Biopharmaceuticals using a Novel Cell-  
Based Assay Platform Technology*

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# Regulatory Perspective on Monitoring for Anti-Drug-Antibodies

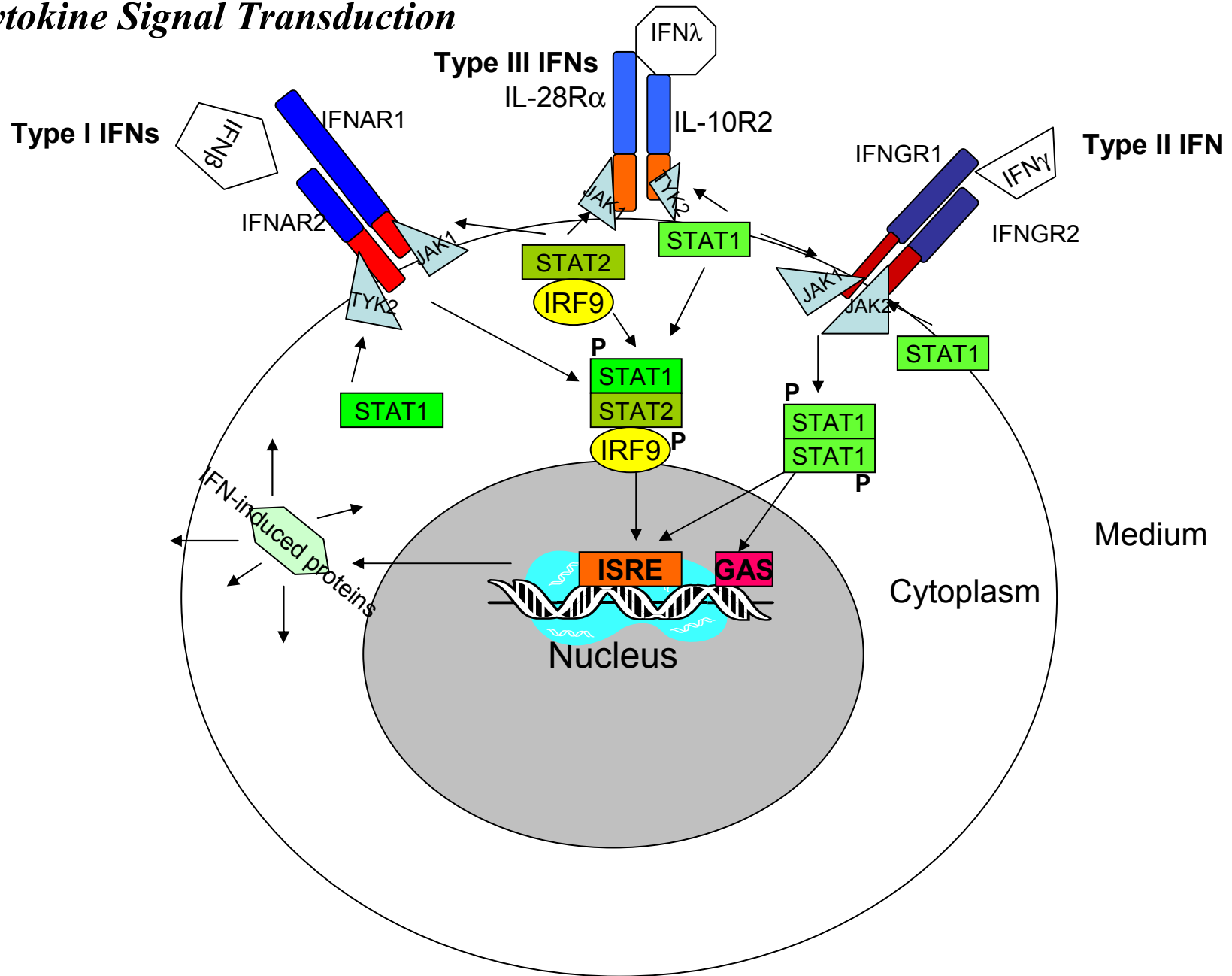
- Monitoring should be clinically driven
- PK data must be analyzed in conjunction with immunogenicity assays
- Cell-based assays should be used to detect neutralizing antibodies *when ever possible*
- Inhibition of receptor binding does not *always* equate to neutralization of biological activity. Abs can neutralize biological activity without inhibiting receptor binding

## *iLite*<sup>TM</sup> Cell Based Assays for the Quantification of Biopharmaceuticals: Objectives

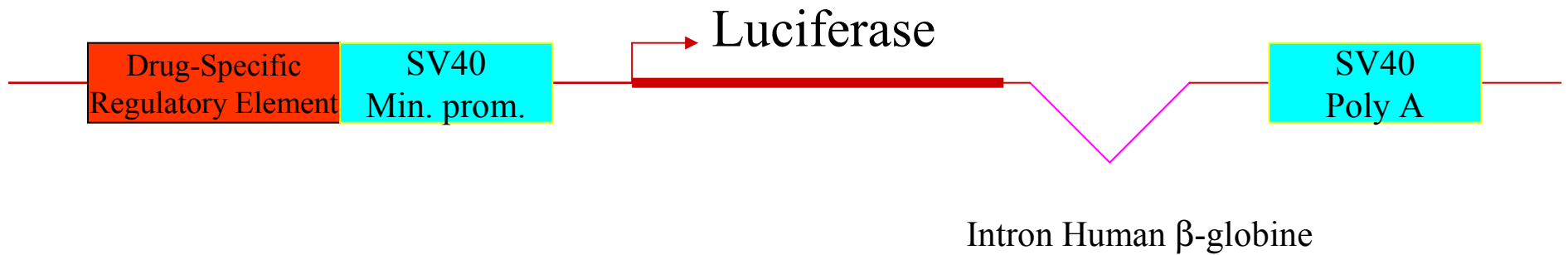
- Replace diverse complex biological endpoints with a single common endpoint
- Eliminate assay variation due to genetic & epigenetic changes associated with continuous cultivation of cells *in vitro*
- Translate Biopharmaceutical Industry Best Practice into the Development of Cell-Based Assays

Quantify Activity Without  
the Need for Live Cells or Cell-Culture

# Cytokine Signal Transduction



# Reporter-Gene Assay: Construction



## *iLite*<sup>™</sup> Reporter-Gene Assays: Strategy

- Stable Transfectant
- Sub-clone
- Master cell bank
- Working cell bank
- Defined cell replication step
- Chemical Treatment
- Cryo-preservation
- Manufactured under ISO 13485 (cGMP)

## *iLite*<sup>TM</sup> Reporter-Gene Assays

- Rapid and precise
- No cell culture
- Obviates assay variation associated with cell growth
- > 3 year stability – 80°C
- Fully automated HTS
- No specialized facilities required
- Facilitates transfer to CRO



## *Neutralization Assay Performance: Clinical Implications*

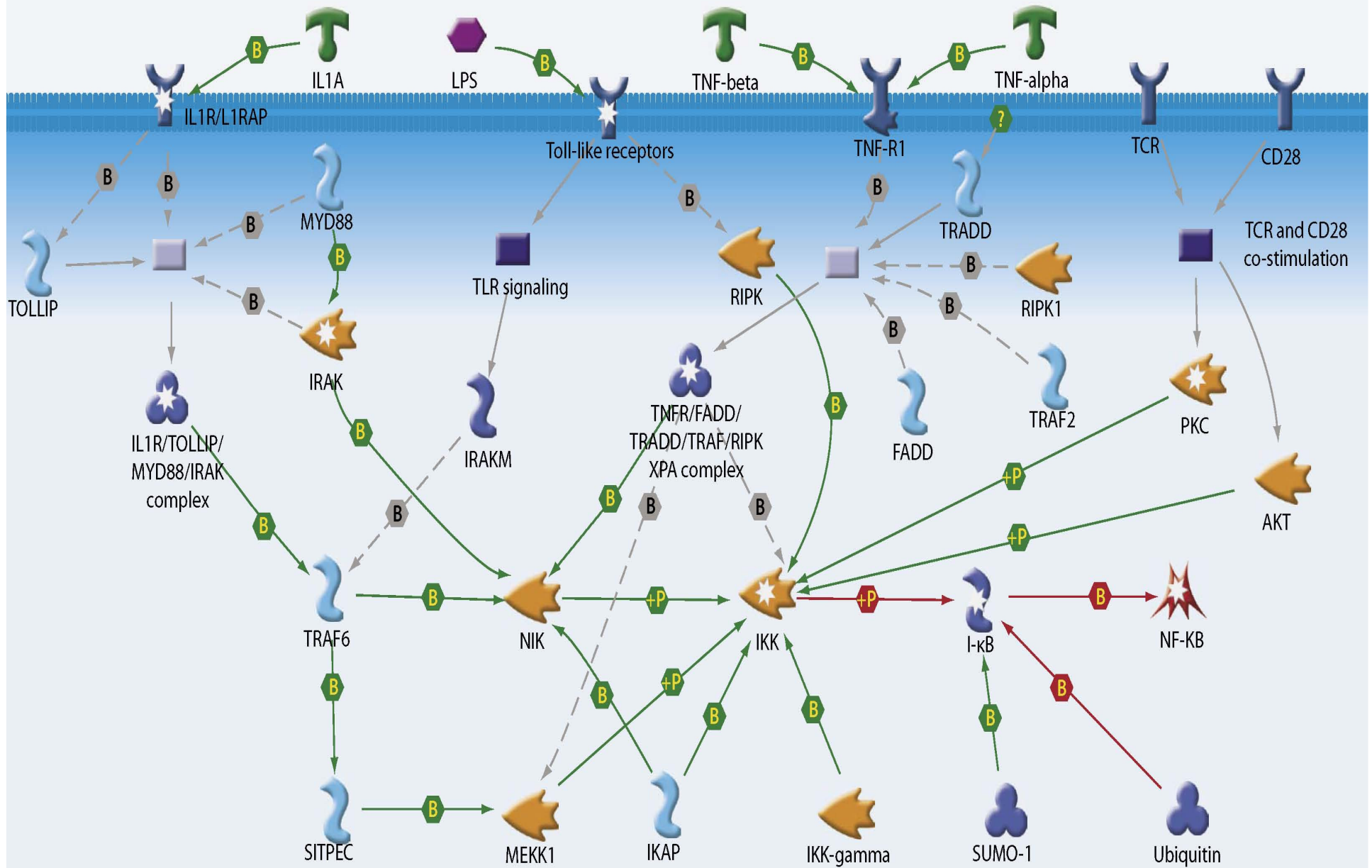
- Sensitivity of assay determines minimum Nab cutoff value
- Low Nab titer : false negative using insensitive assay
- *iLite* neutralization assay superior sensitivity to use of live cells

# Quantification of Neutralizing Antibodies to TNF- $\alpha$ Antagonists

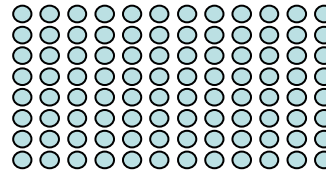
# Detection of antibodies to TNF- $\alpha$ antagonists

- TNF- $\alpha$  signals through NF $\kappa$ B
- Numerous other cytokines:  
IL1- $\beta$ , IL-2, IL-4, IL-10, IL-18, IFN  $\beta$ , etc.  
also employ the NF $\kappa$ B pathway

# NF-κB Signaling Pathway

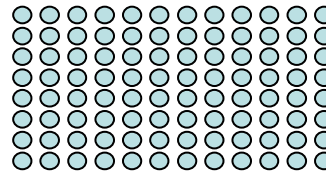


TNF alpha Antagonist  
10 LU/ml



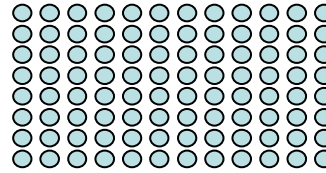
Dilutions  
10<sup>-1</sup>  
10<sup>-2</sup>  
10<sup>-3</sup>  
10<sup>-4</sup>  
10<sup>-5</sup>  
10<sup>-6</sup>  
10<sup>-7</sup>  
10<sup>-8</sup>

Serum samples  
In serial Dilution



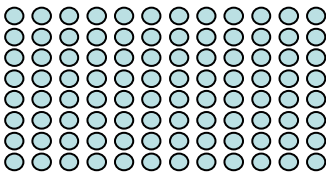
10'

TNF alpha  
10 LU/ml



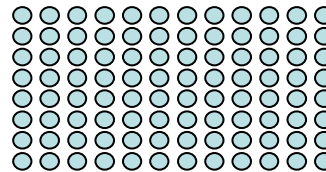
10'

Reporter  
Cells



5 h

Britelite



Reading

Controls:  
Cells +Serum alone  
Cells +Serum + TNF

# *iLite*<sup>TM</sup>: Detection of TNF- $\alpha$ in Sera from Patients with Rheumatoid Arthritis

- 0/112 Samples analyzed exhibited detectable levels of TNF- $\alpha$

# *iLite*<sup>TM</sup> Quantification of Neutralizing antibodies to TNF- $\alpha$ Antagonists

- Robust assay no interference from circulating levels of TNF- $\alpha$  (like) activity
- Readily detect circulating levels of TNF- $\alpha$  antagonists (activity)
- Readily detect NAbs to TNF- $\alpha$  antagonists
- Readily distinguish between NAbs to different TNF- $\alpha$  antagonists
- A single assay for all TNF- $\alpha$  antagonists, allows direct comparisons of relative immunogenicity of drugs, including novel drugs in development or biosimilars

Anti-TNF Sera							
Serum #	Patient #	TNF-alpha Antagonist			Current Treatment		
		Etanercept	Adalimumab	Inflixab			
		Enbrel	Humira	Remicade			
1	0123247			4			np
2	0124063	12	12	24			np
3	0124064		30				Rituximab (Mabthera)
4	0130022	14	15	24			Rituximab (Mabthera)
5	0207179	18	6				
6	0208007		6	3			Rituximab (Mabthera)
7	0208014						None
		Duration of treatment in months					



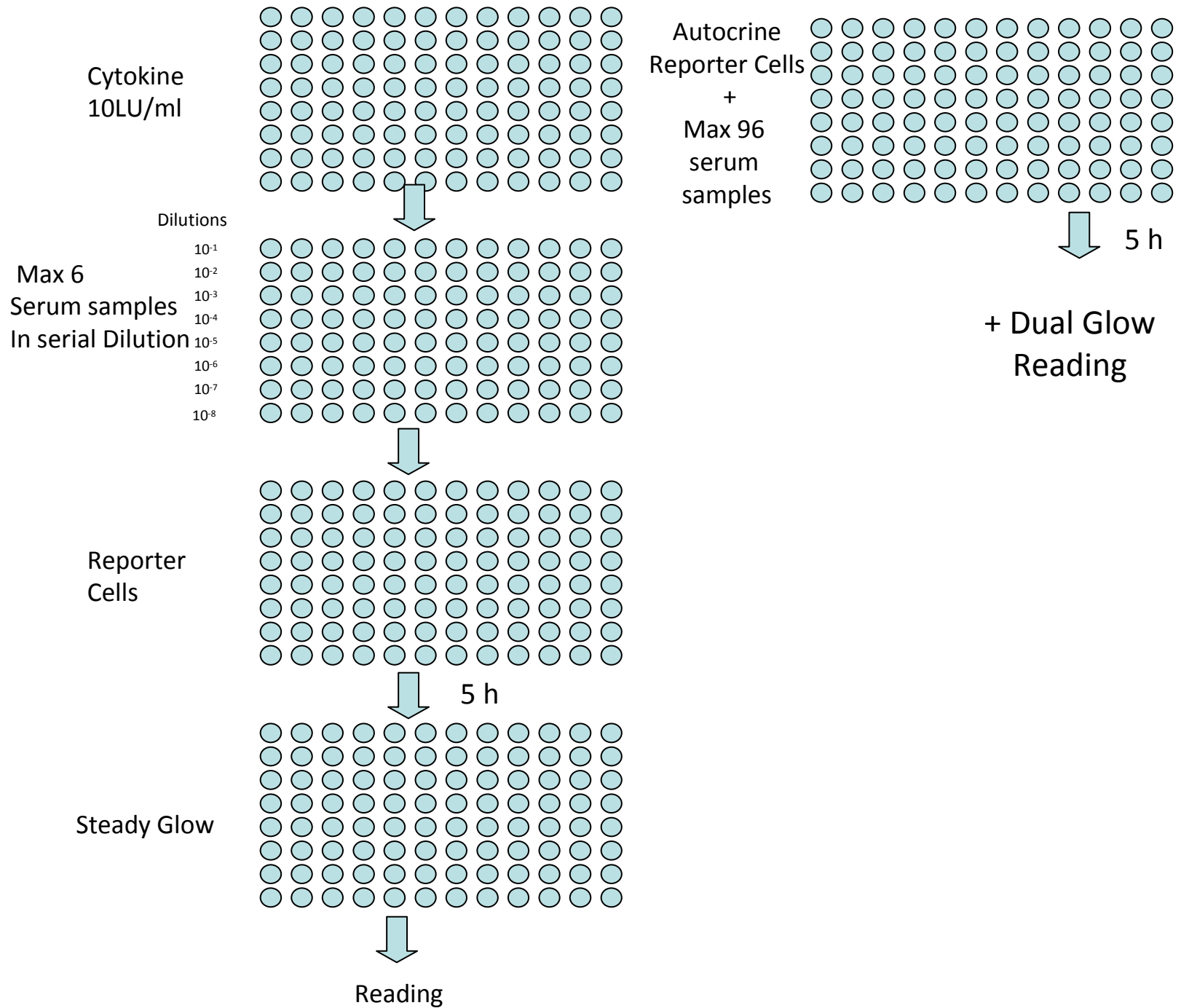
## *iLite*<sup>™</sup> Quantification of Neutralizing antibodies to Anti-inflammatory Biologicals

- Simultaneous quantification of drug and anti-drug NAbs in the same assay
- A common assay format for TNF- $\alpha$  antagonists, anti-CD20 Mabs or other anti-inflammatory biologicals
- Readily distinguish between NAbs to different TNF- $\alpha$  antagonists
- A common assay read-out for TNF- $\alpha$  antagonists, anti-CD20 Mabs and other anti-inflammatory biologicals allows direct comparisons of relative immunogenicity of drugs.

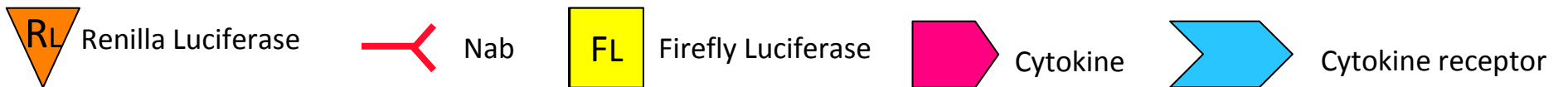
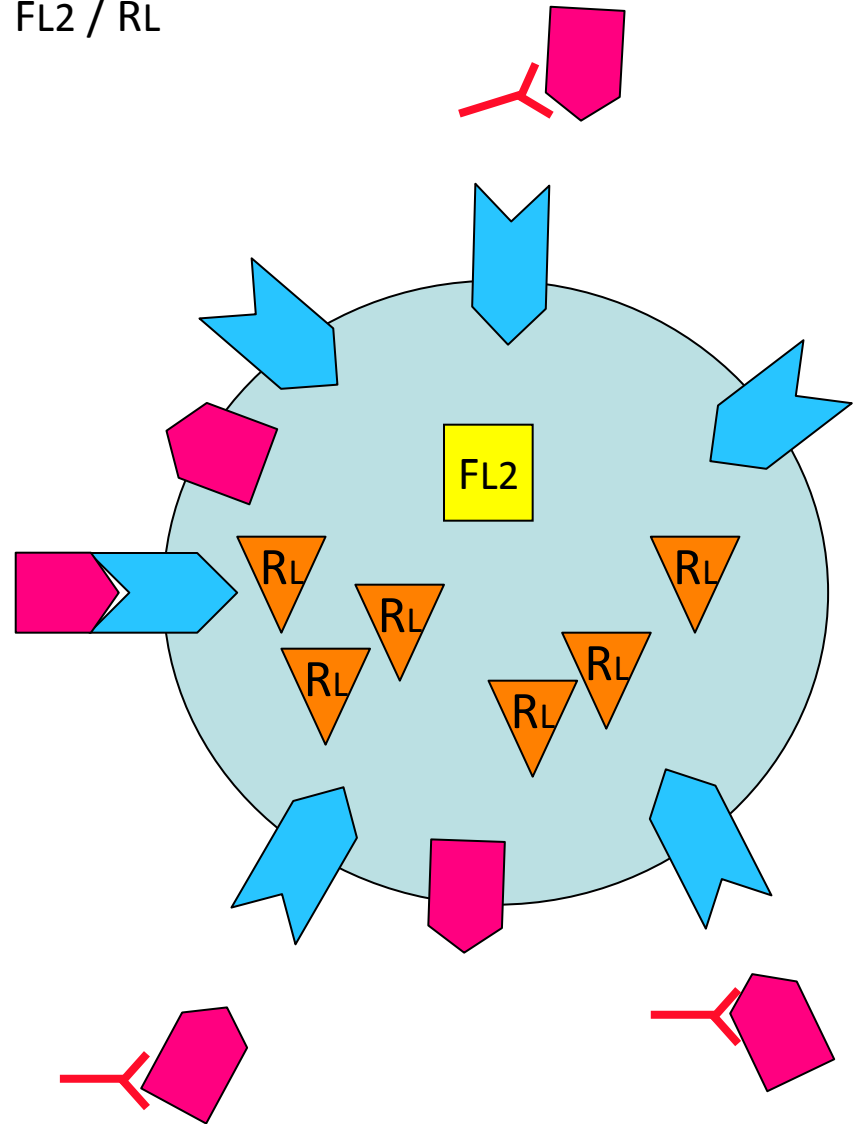
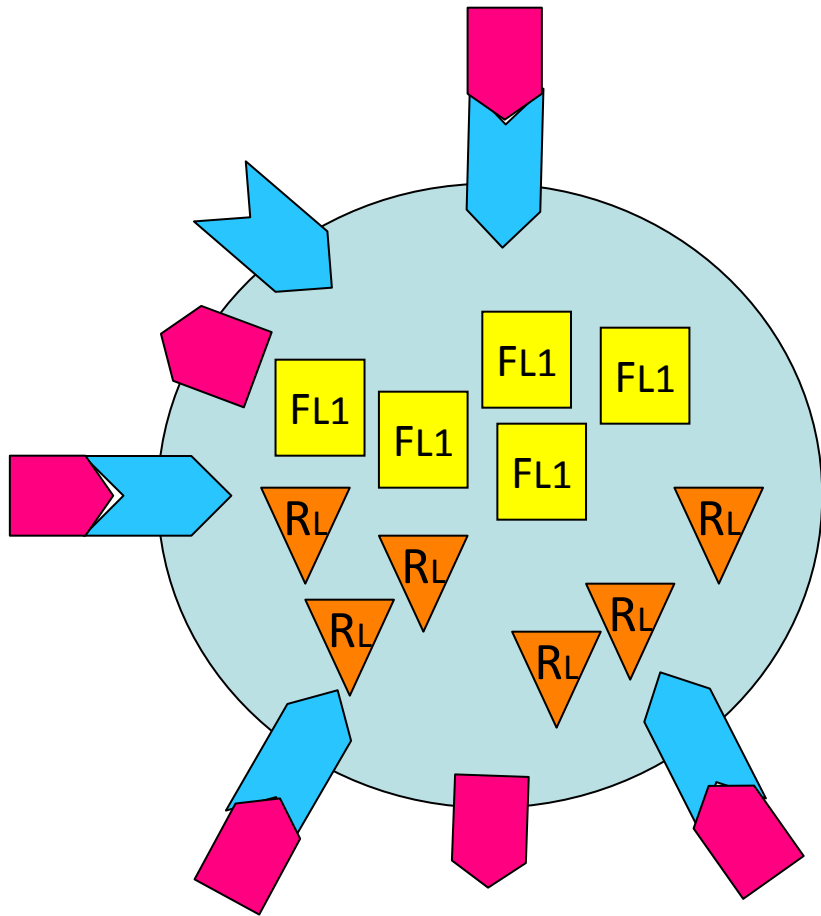
*iLite*<sup>™</sup> Quantification of anti-Drug NAbs :

Future Developments

# One-Step *iLite*<sup>™</sup> Neutralization Assay



FL1 / RL > FL2 / RL



## *One-Step* Nab Assay

- Quantification of circulating drug levels
- No sample manipulation
- No drug standard curve required
- No control samples required
- High degree of precision results normalized relative to internal control; CV 4 to 8%
- Results independent of cell number
- Results independent of serum matrix effects
- Ideally suited to high through-put
- Reduced assay time

## *iLite*<sup>™</sup> Technology

- Fast, easy, reliable
- Excellent lot-to-lot, day-to-day repeatability/reproducibility
- 3 years of market experience (Biogen Idec, Merck-Serono, BMS, Schering-Plough and others)
- Issued US Patent (USPN 7,740,556) and several pending applications
- ISO13485 audited, certified manufacturing facility (Biomonitor Ltd, Galway Ireland).

## Current Products (**Biomonitor A/S**)

### USE

<i>iLite</i> <sup>TM</sup> alphabeta	Detection and quantification of Type I human IFNs (RUO).
<i>iLite</i> <sup>TM</sup> antibeta	Detection and quantification of NAbs in sera of MS patients being treated with IFN-beta (RUO).
<i>iLite</i> <sup>TM</sup> antialpha	Detection and quantification of NAbs in sera of patients being treated with IFN-alpha (RUO).
<i>iLite</i> <sup>TM</sup> alphabeta CE	CE Marked assay for the detection and quantification of IFN-alpha in patient sera to replace the CPE assay as an aid to the physician in optimizing therapy.



# Products in Development (**Biomonitor A/S**)

## USE

*iLite*<sup>TM</sup>anti-infliximab *CE*

CE Marked assay for the detection and measurement of NAbs produced against commercial formulations of infliximab in patient sera.

*iLite*<sup>TM</sup>anti-etanercept *CE*

CE Marked assay for the detection and measurement of NAbs produced against commercial formulations of etanercept in patient sera.

*iLite*<sup>TM</sup>anti-adalimumab *CE*

CE Marked assay for the detection and measurement of NAbs produced against commercial formulation of adalimumab in patient sera.