Tired of ELISA? – It's Time for Lumit™ Immunoassays

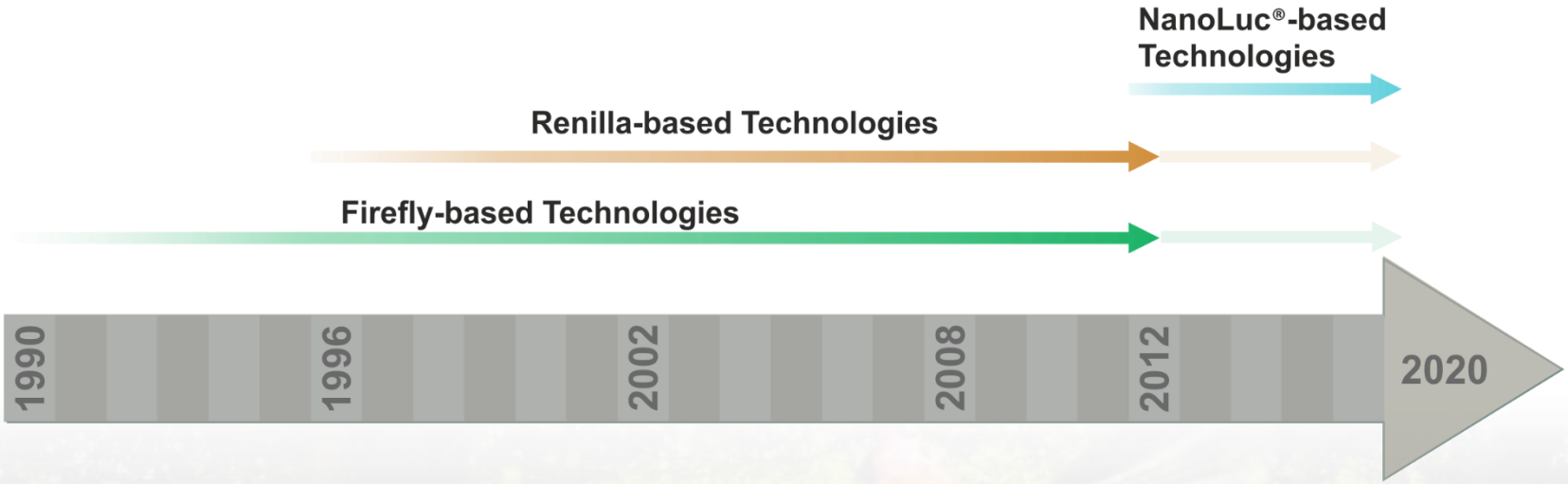
An Easier and Faster Method for Protein Detection

Dr. Erik Bonke | Application Specialist | Promega GmbH

Welcome

Promega – The Bioluminescent Company

A Continuously Grown Expertise in Luciferase-based Technologies



- Reporter Gene Assays
- GloSensor™ (cAMP, Protease Assays)
- GloResponse™ (Signaling Pathways)
- Rapid Response™ (Signaling Pathways)
- Cell-Health Assays
- Bioassays (ADCC, PDL1)
- NanoBRET™ / NanoBiT® (Protein Interaction)
- NanoBRET™ Target Engagement
- HiBiT Protein Tagging System
- Lumit™ Immunoassays

Lumit™ Immunoassays

The Powerful Alternative to Conventional Immunoassay Approaches

Traditional ELISA Workflow

- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps

Lumit™ Immunoassays

The Powerful Alternative to Conventional Immunoassay Approaches

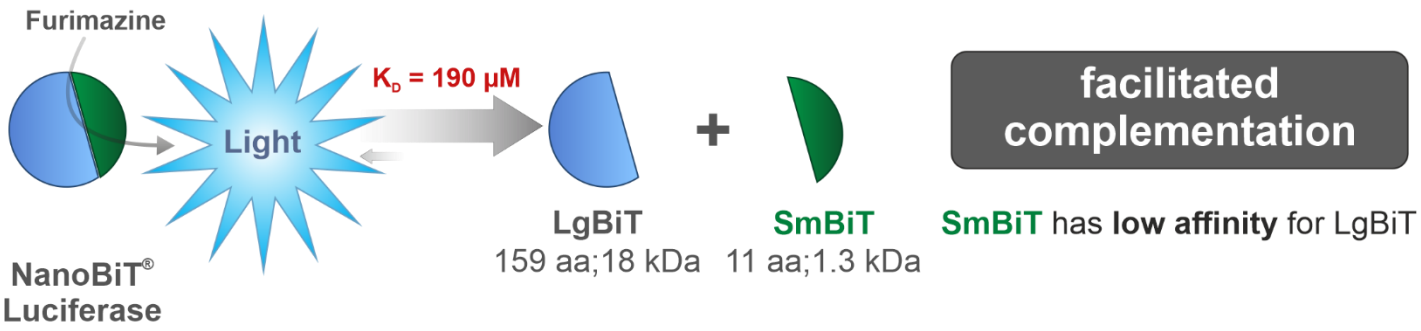
Traditional ELISA Workflow

- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps
- Based on NanoLuc® luciferase we developed *Lumit™ Immunoassays*
  - ✓ Easy and fast (30 – 120 min)
  - ✓ High Sensitivity (low number of cells)
  - ✓ Broad dynamic range (3 – 4 logs)
  - ✓ Flexible formats (96- or 384-well)
  - ✓ Homogenous and HTS compatible

Hwang, B. *et al.* (2020) Commun Biol. 3:8

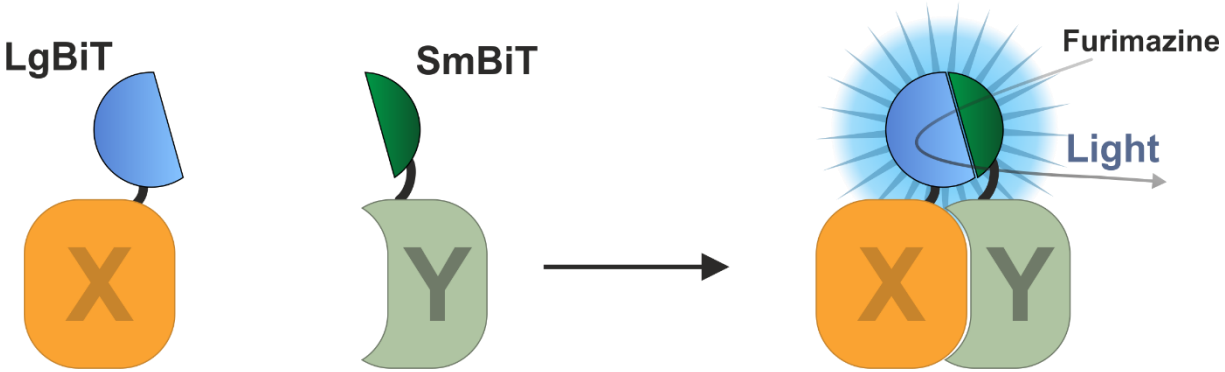
NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies



Complementation facilitated through … (direct) protein:protein interaction → NanoBiT® PPI System

Dixon *et al*. 2016, *ACS Chemical Biology*



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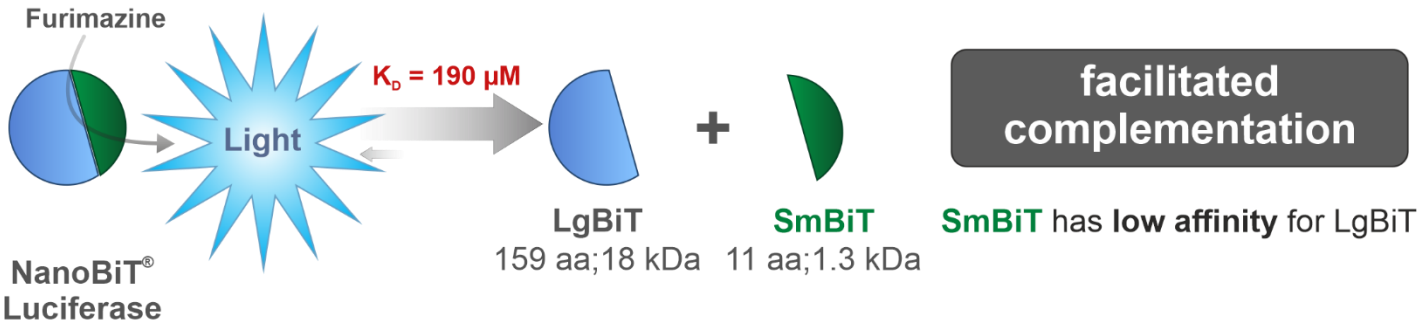
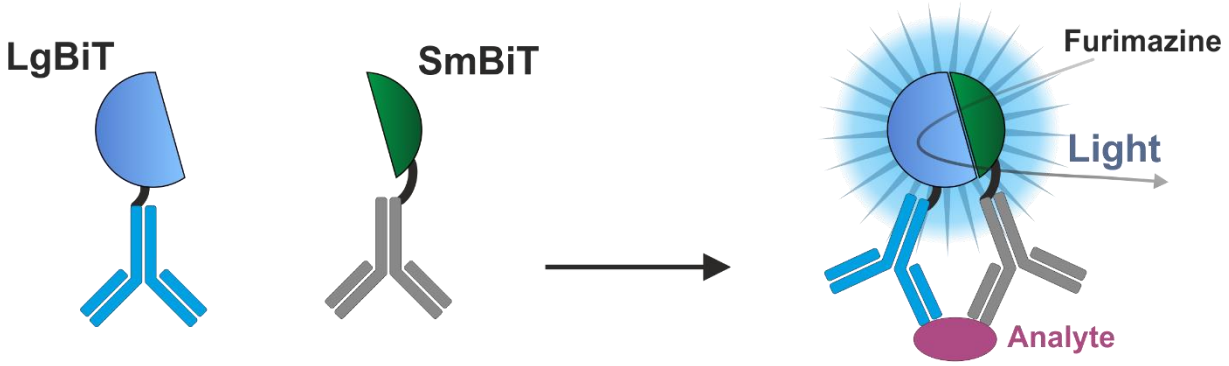


Complementation facilitated through … (indirect) Ab:Ab "interaction" → Lumit™ Immunoassays

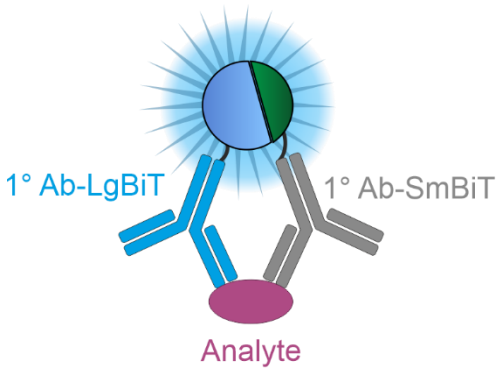
Hwang *et al*. 2020, *Commun Biol.*



Lumit™ Immunoassays

Different Formats for Maximum Flexibility

Direct



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- Validated for cytokines and peptide hormones
- *Ready-to-use* assays for
  - ✓ IL1-β, IFN-γ, IL-2, IL-6, IL-10, IL-4, TNF-α, VEGF, …
  - ✓ Insulin and glucagon

Indirect

- Avoids labeling of 1°Abs
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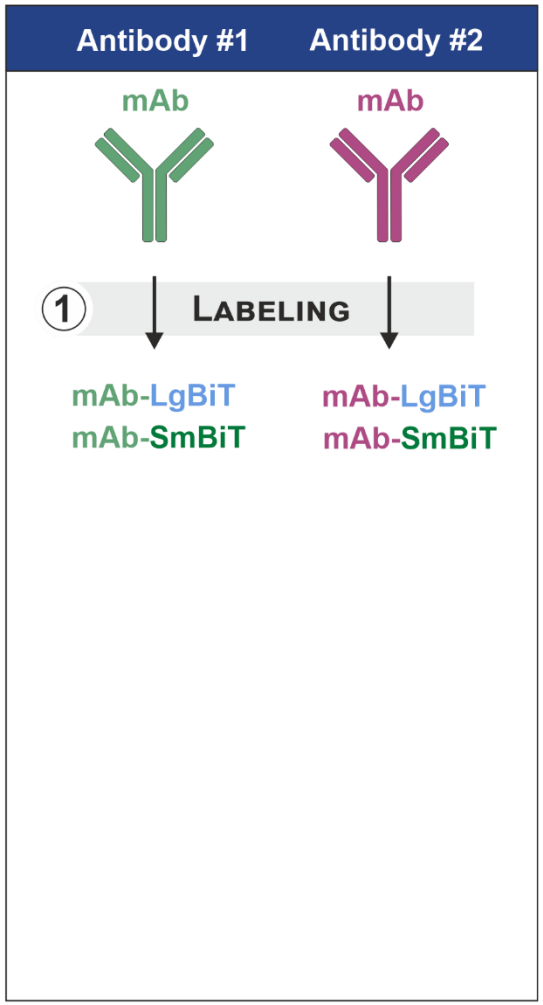
Competitive

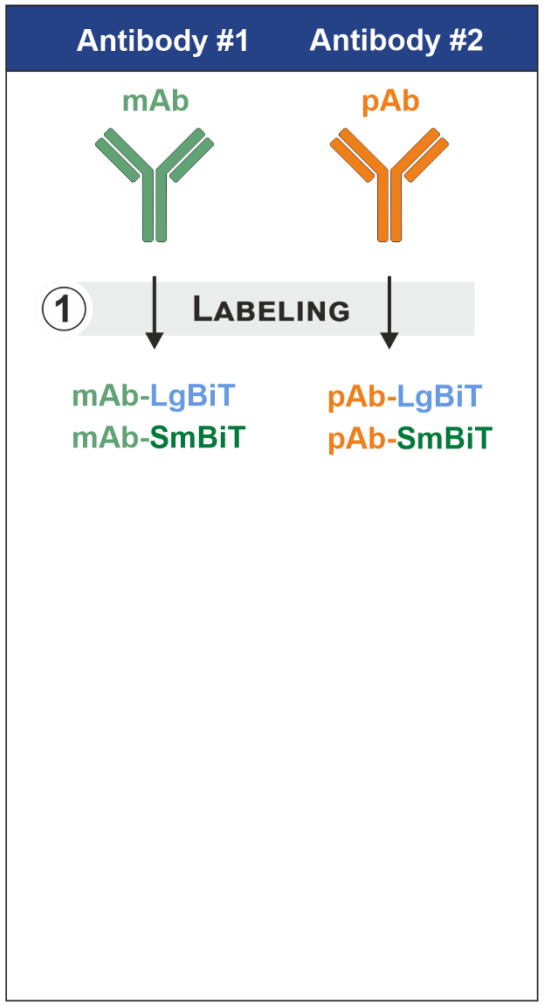
- Requires antibody and tracer labeling
- Establish competitive antibody binding assays
- *Ready-to-use* assay for hFcRn:Fc protein interactions, e.g. antibodies
  - ✓ Lumit™ FcRn Binding Immunoassay

Build-Your-Own Direct Lumit™ Immunoassay

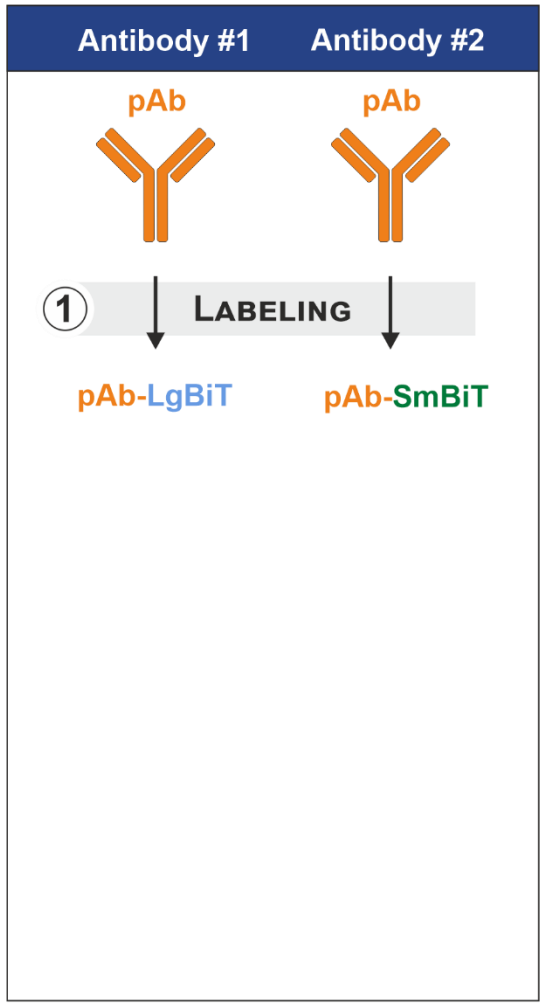
Various Options for Your Convenience



☺ highest specificity and sensitivity



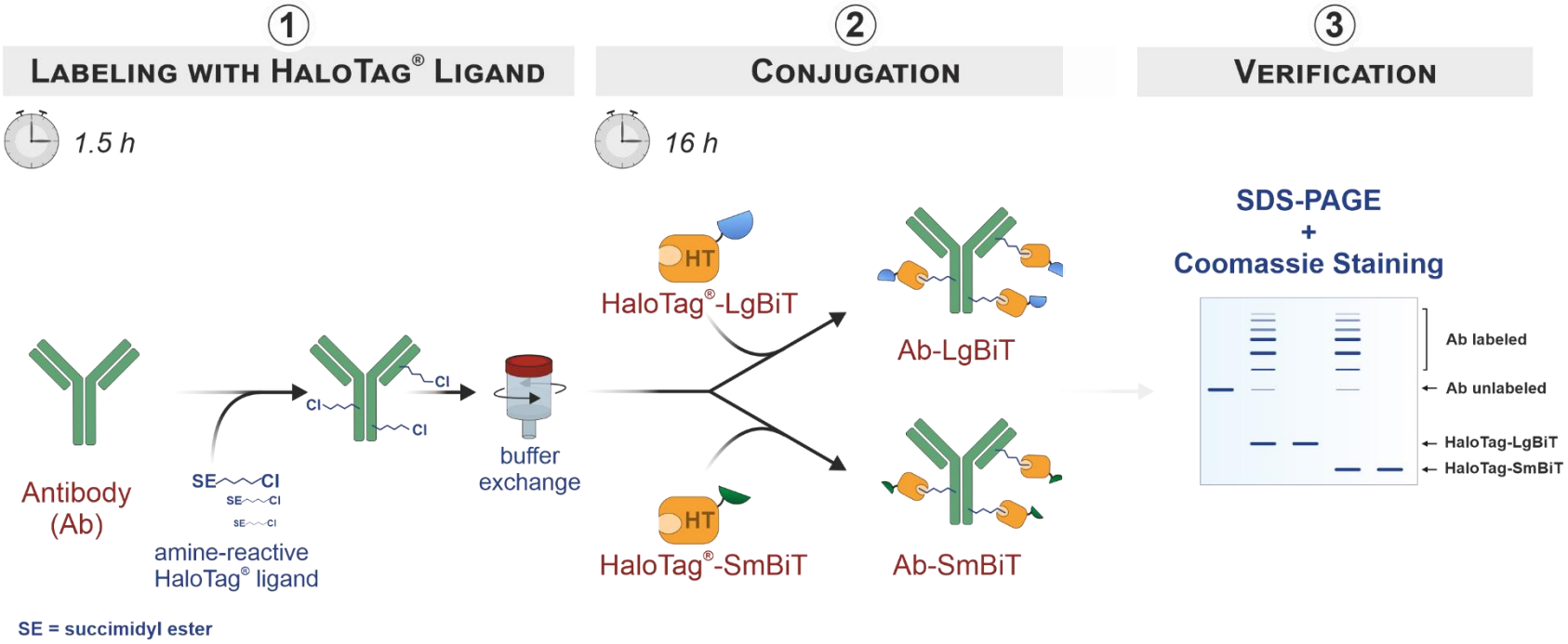
☺ only one mAb needed



☺ most inexpensive variant

Build-Your-Own Direct Lumit™ Immunoassay

Step 1: Labeling of Antibodies with the Lumit™ Immunoassay Labeling Kit



FACTS

- Easy and robust 2-day protocol
- Attachment is highly efficient (> 90%)
- Oriented BiT subunits for maximum activity

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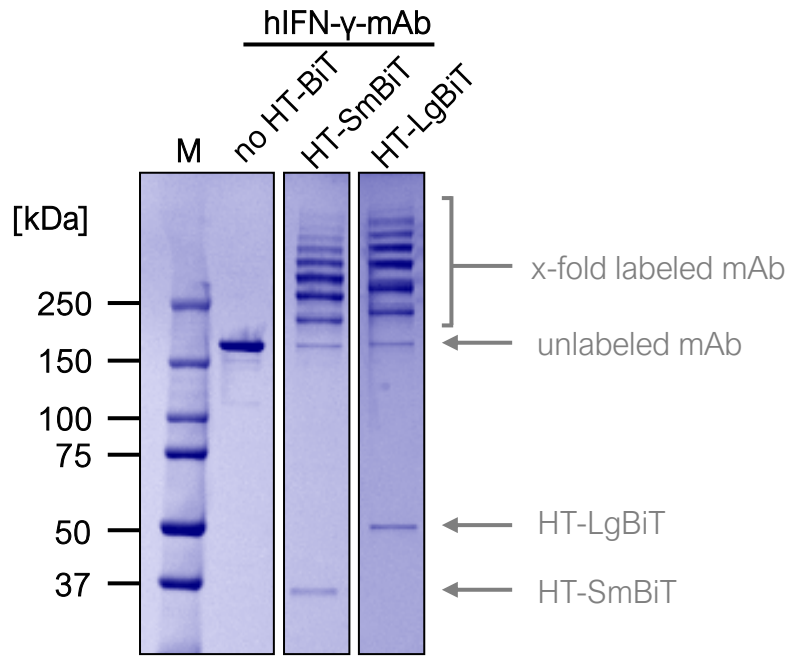

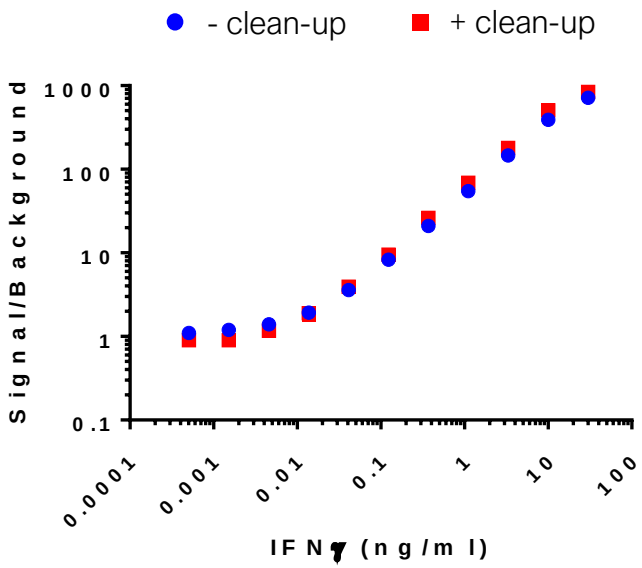

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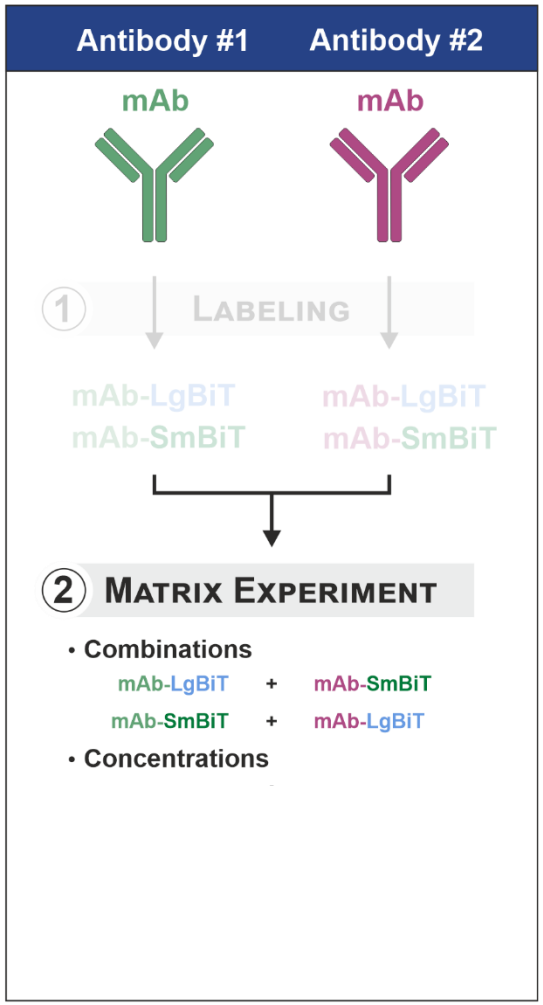

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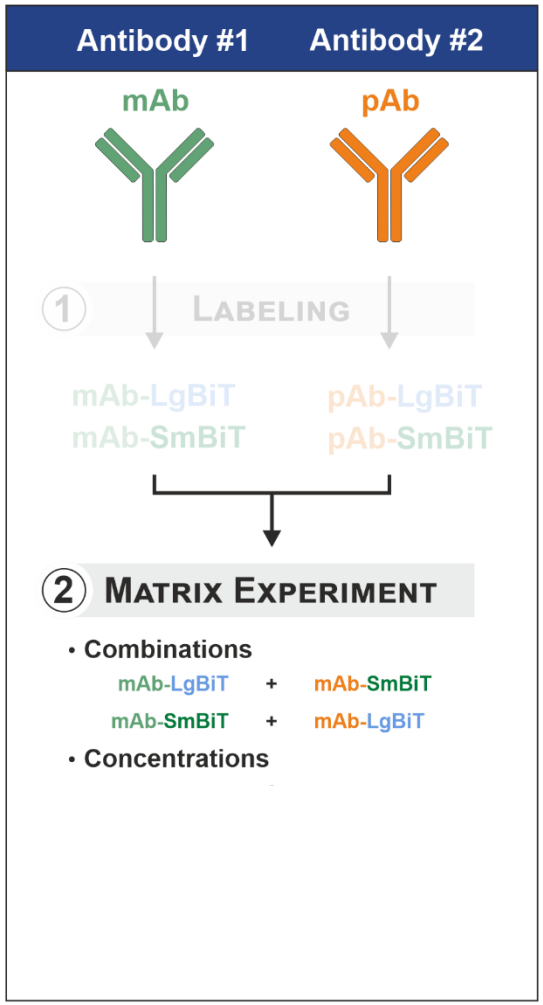

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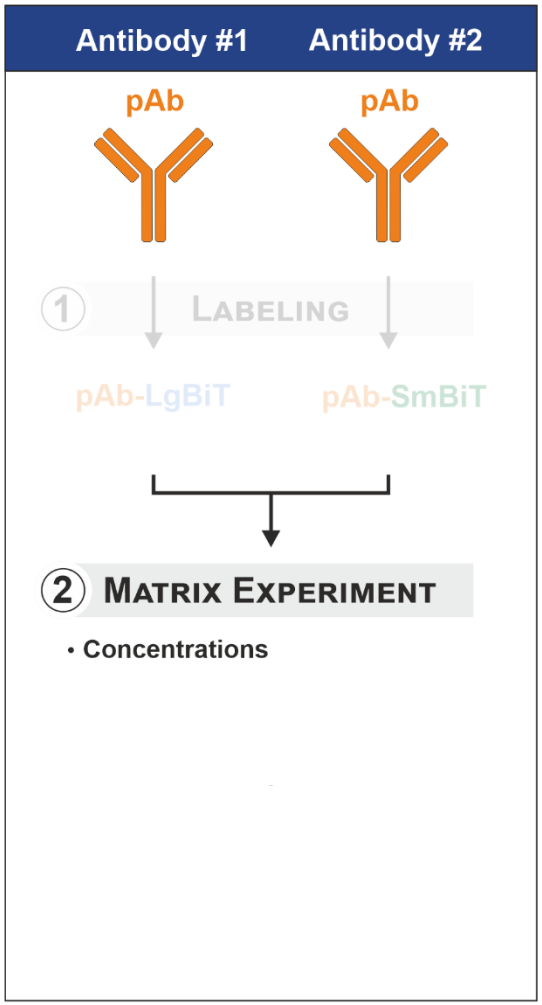

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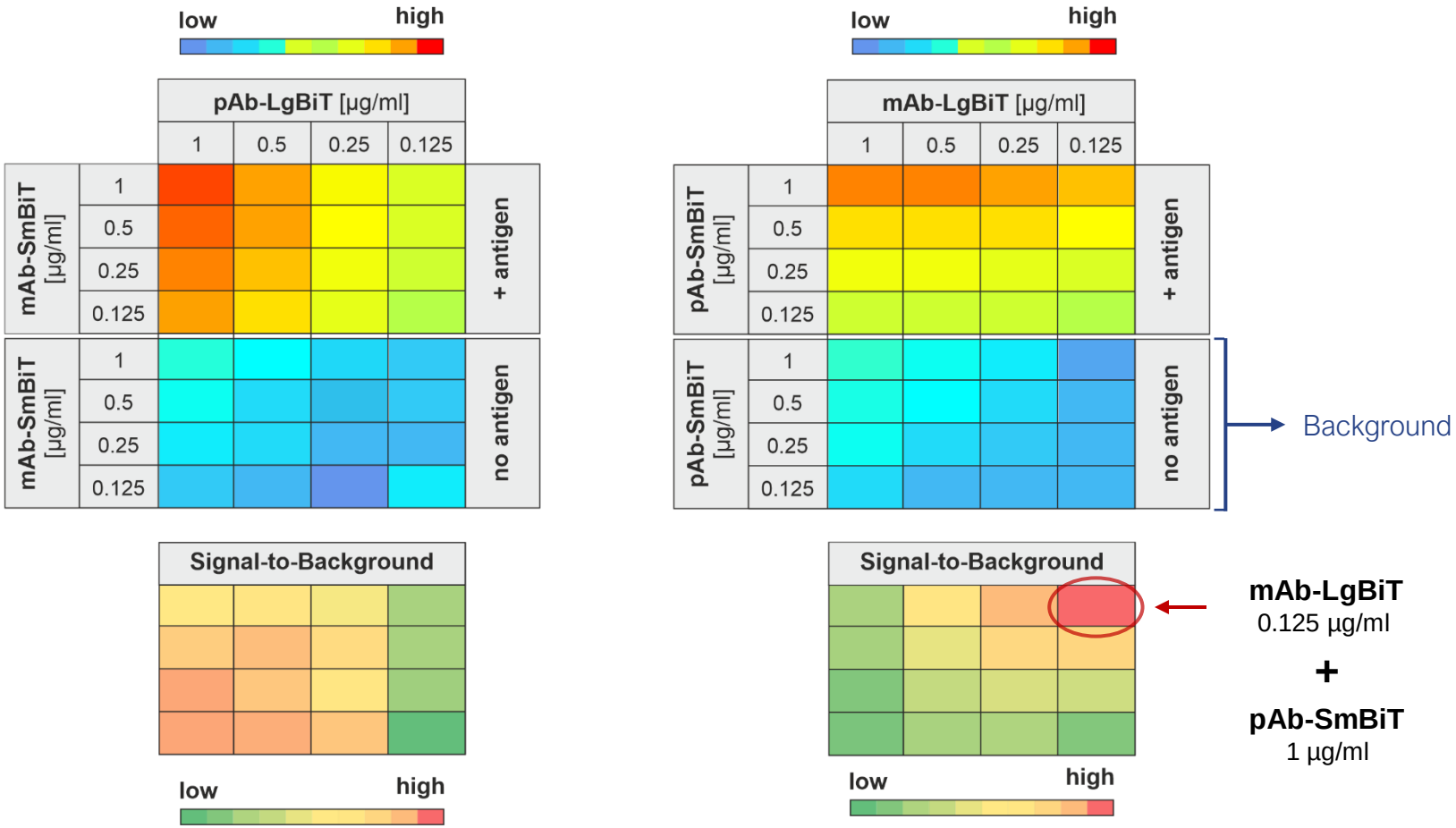
Step 2: Identification of best antibody combination/concentration

Matrix experiment to determine maximal signal-to-background (S/B) ratio



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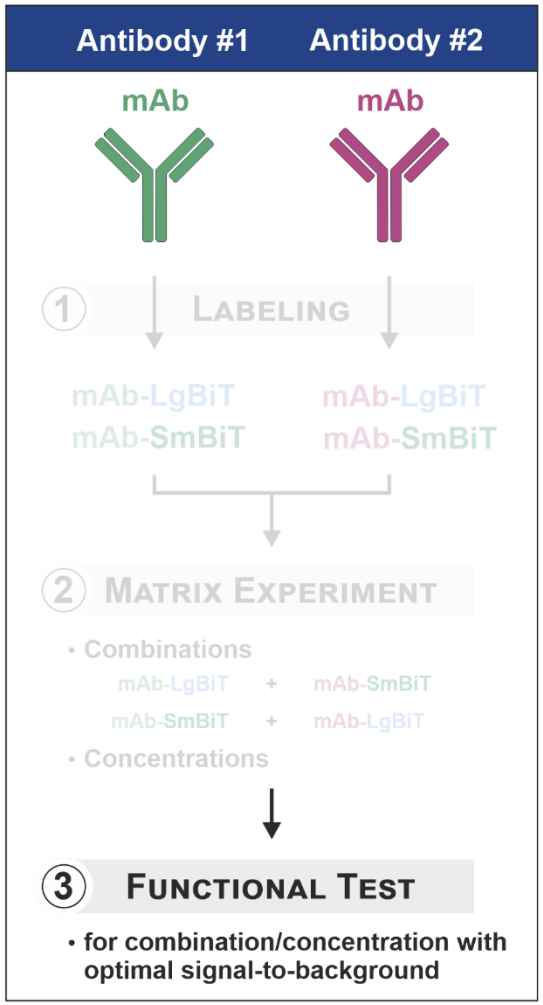

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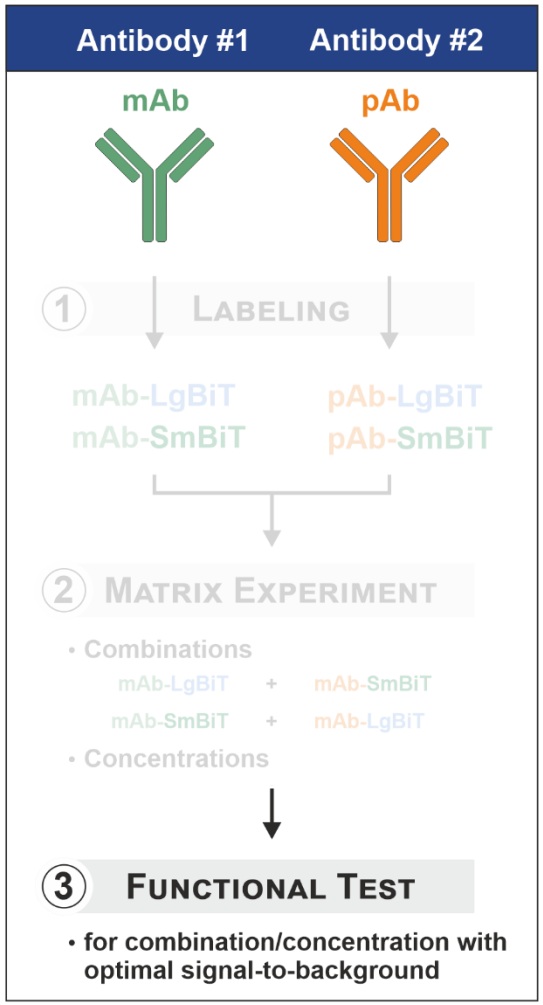

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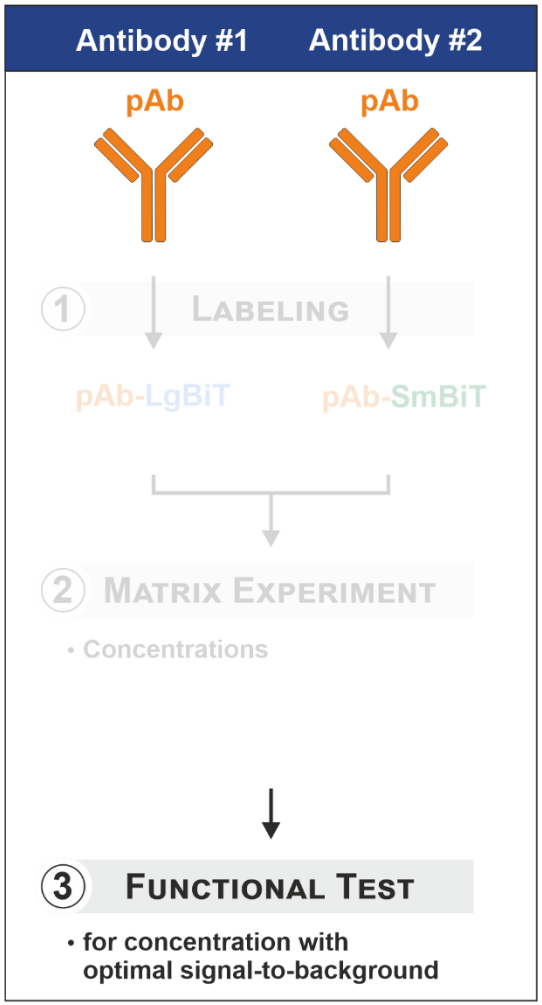

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Build-Your-Own Direct Lumit™ Immunoassay

Step 3: Functional Test of Identified Antibody Combination

- **Treatment of cells**
- **Add standard dilutions**

- **Add NanoBiT®-labeled 1° Ab mixture**

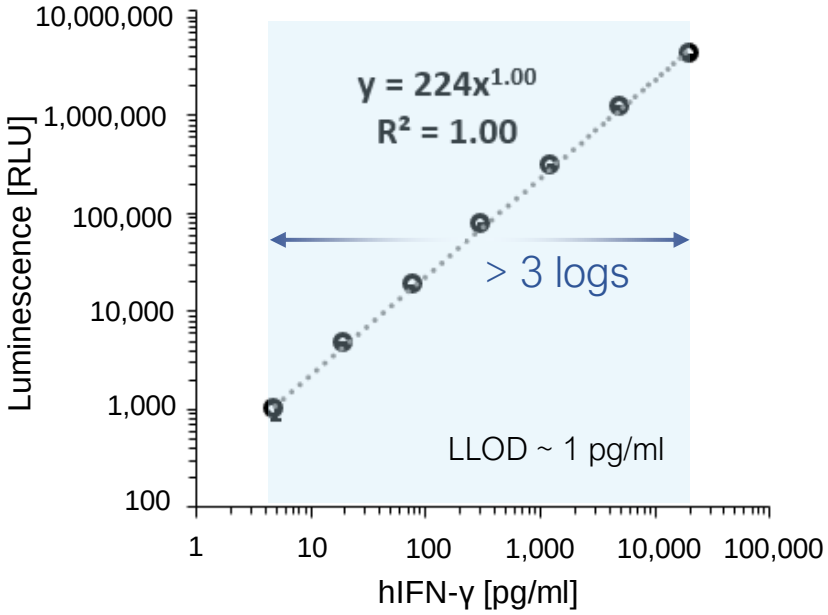
  *directly to standard, cells **or** aliquot of supernatant*

- **Incubate**

- **Add Detection Reagent**

- **Measure Luminescence**

Lumit™ (human) IFN-γ Immunoassay



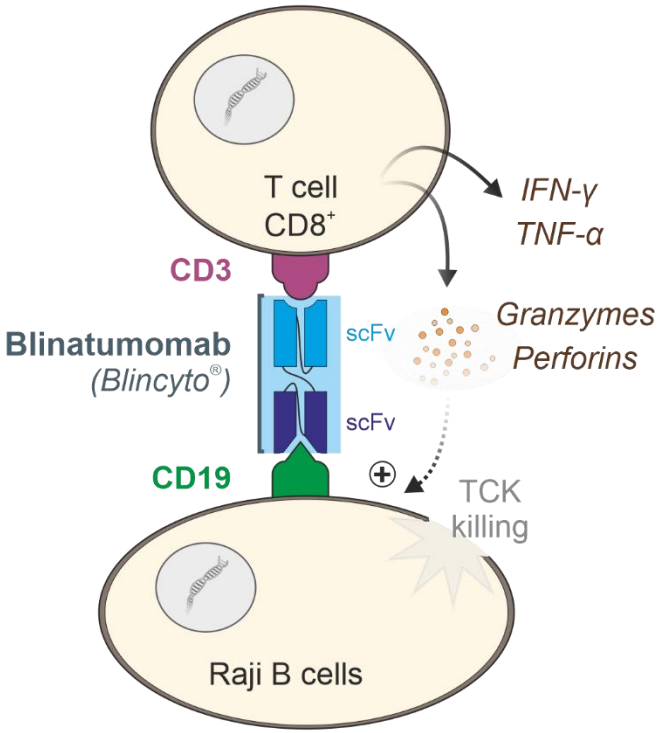
OBSERVATIONS

- Good sensitivity and broad dynamic range (≥ 3 logs)
- No need for sample dilution

Build-Your-Own Direct Lumit™ Immunoassay

Step 3: Functional Test of Identified Antibody Combination

BiTE-induced IFN-γ release from CD8⁺ T cells



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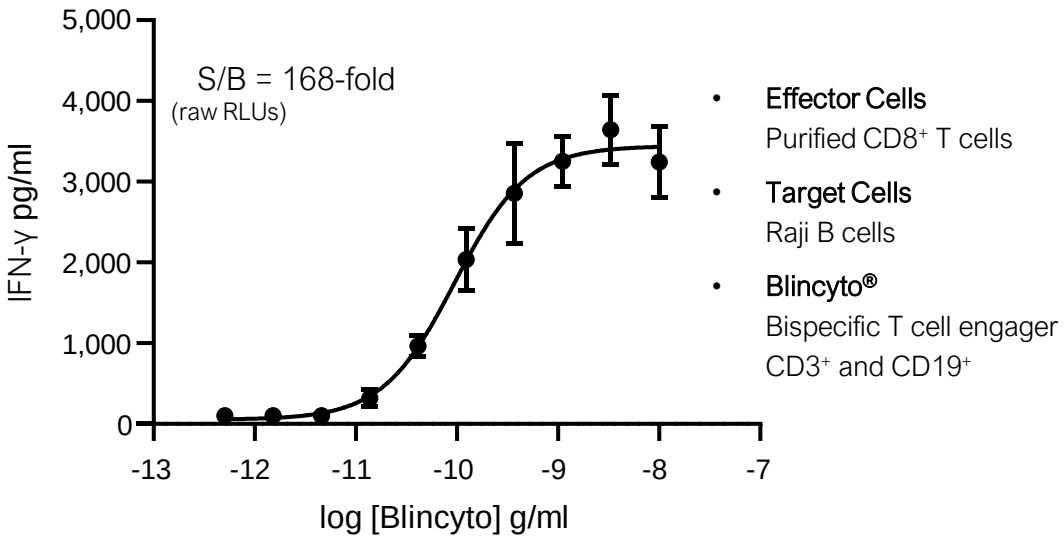

OBSERVATIONS

- Good sensitivity and broad dynamic range (≥ 3 logs)
- No need for sample dilution
- Excellent cell-based performance (addition to cells)

BiTE: Bispecific T cell engager

scFv: single-chain variable fragment

Lumit™ Immunoassays

Different Formats for Maximum Flexibility

Direct

- Requires labeling of 1°Abs
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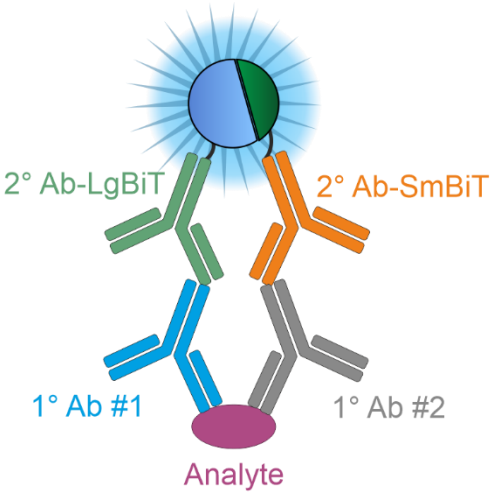

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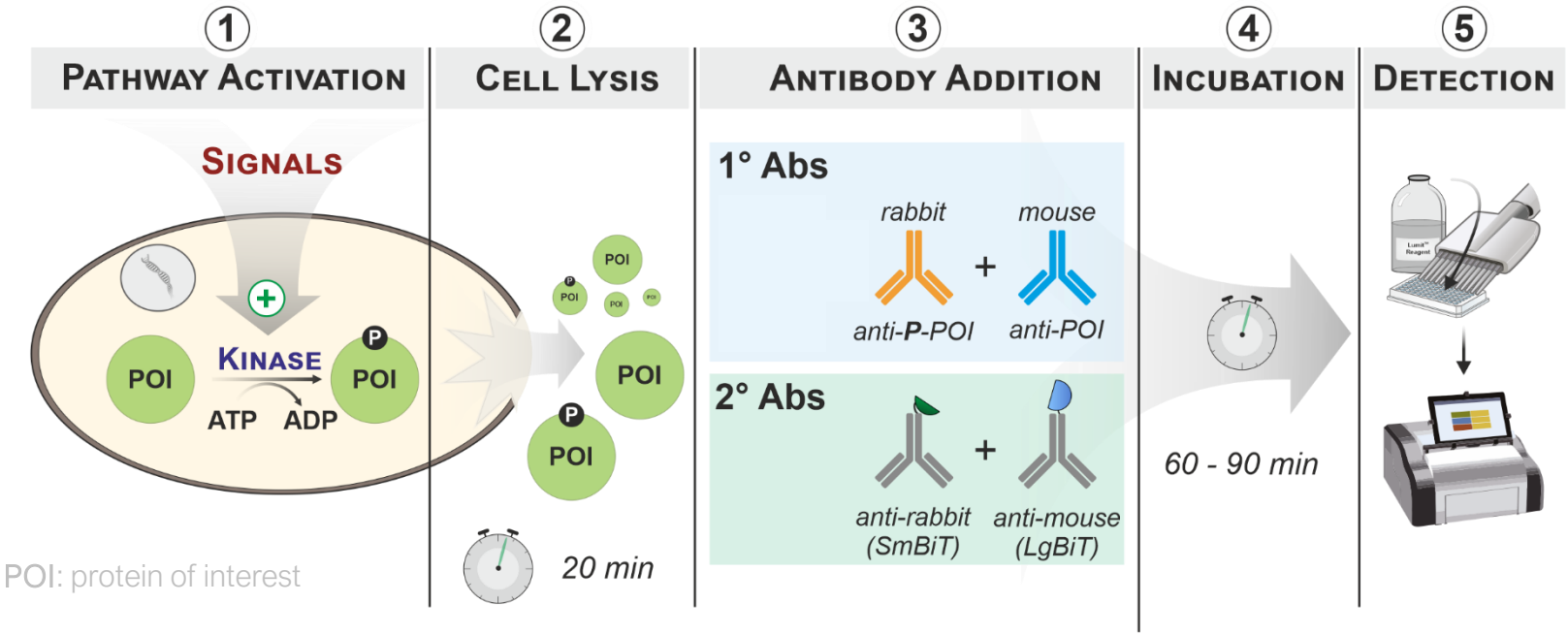
Lumit™ Immunoassay Cellular Systems

Study Cellular Signaling Events



Additionally available pre-labeled 2° Abs:

- anti-rabbit (LgBiT)
- anti-mouse (SmBiT)
- anti-goat (LgBiT)
- anti-goat (SmBiT)

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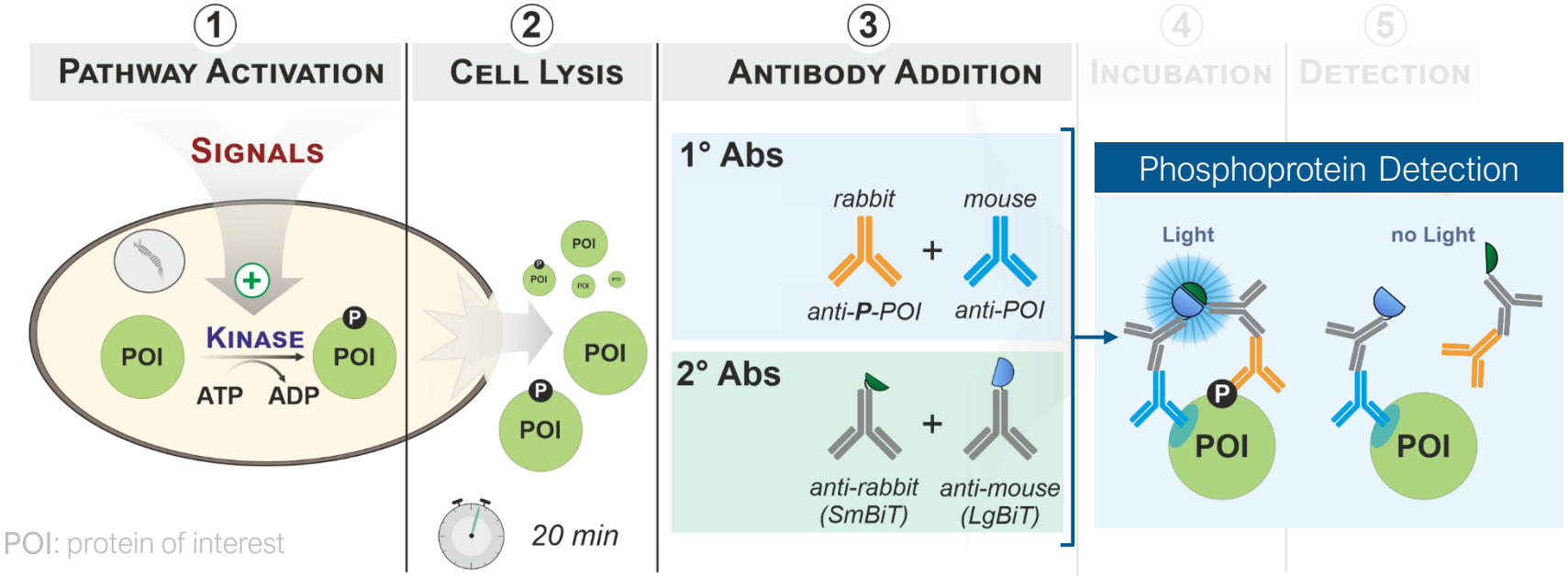

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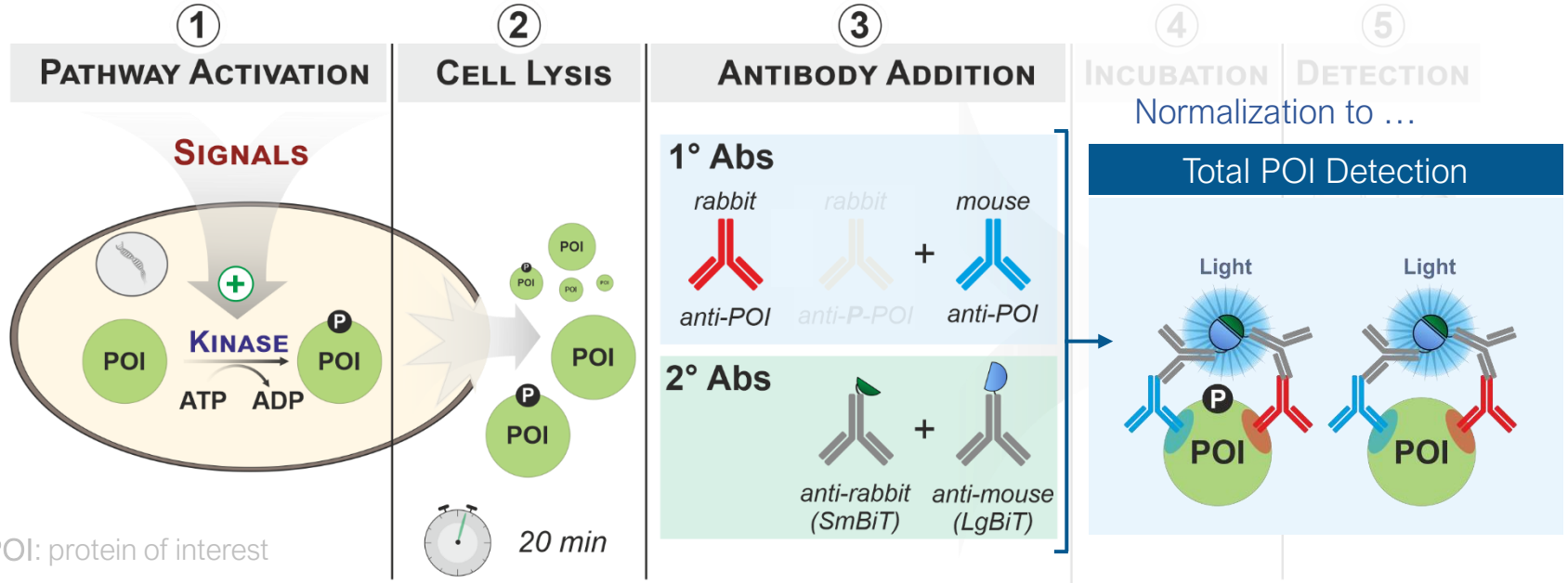
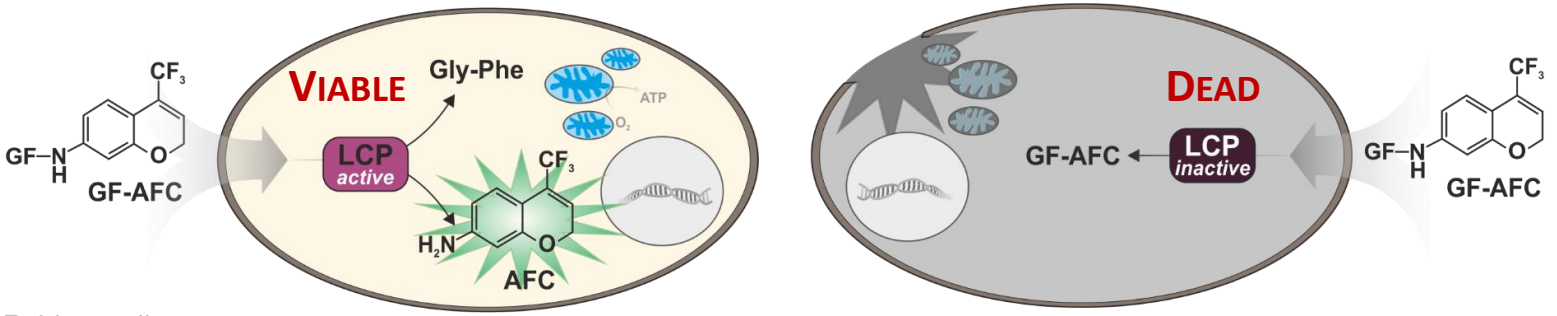


POI: protein of interest

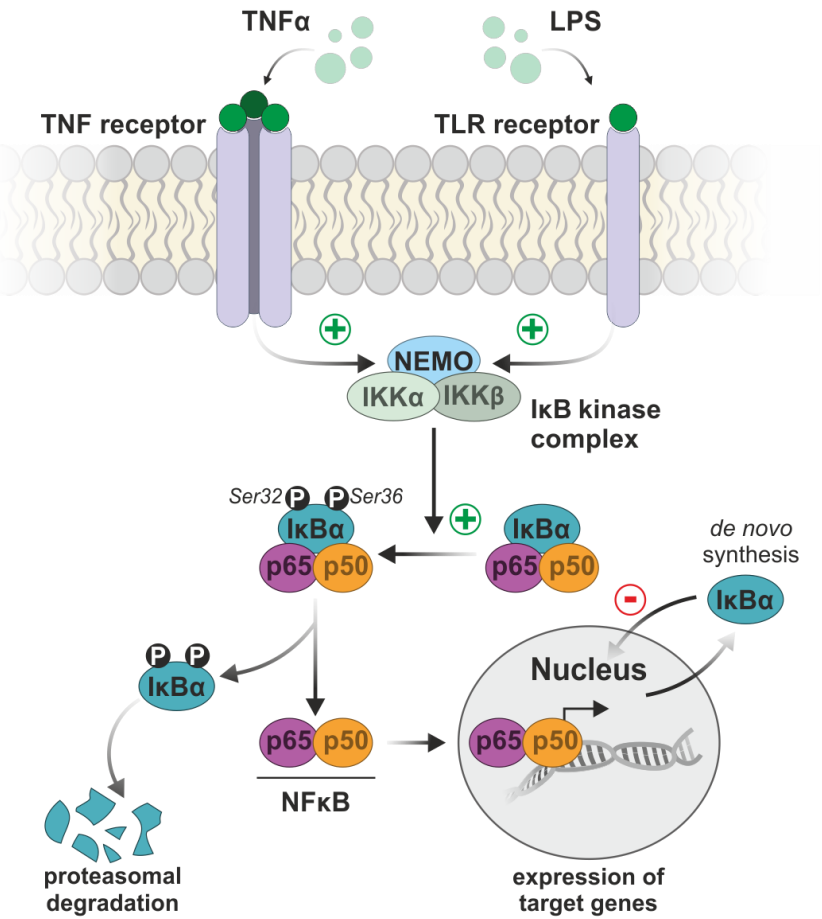
Normalization on number of viable cells using CellTiter-Fluor™

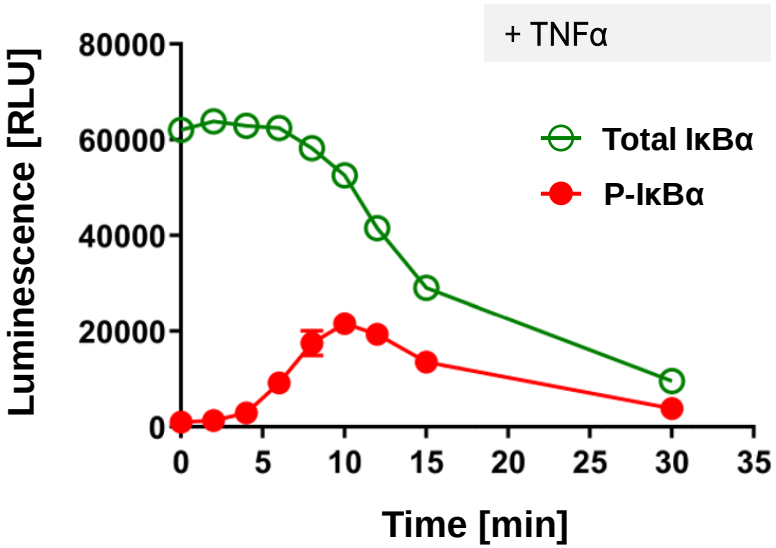


LCP: Live-cell protease

Signaling Pathway and Kinase Activity Analysis

Studying the NFκB Pathway





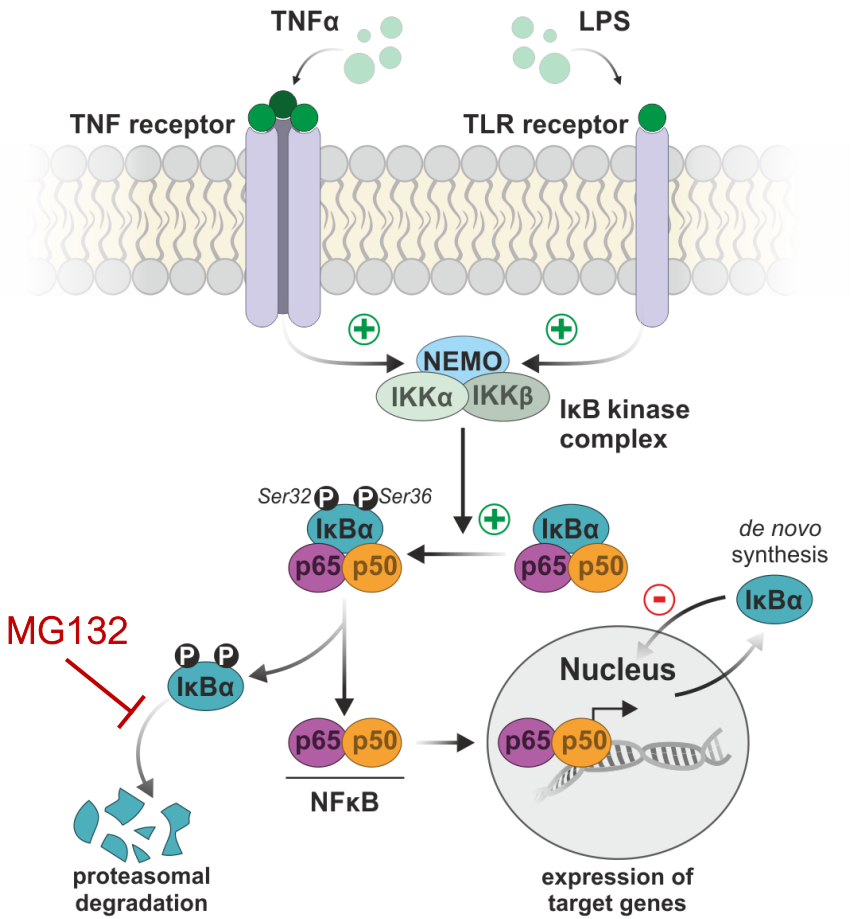
Modified from Hwang, B. *et al.* (2020) Commun Biol. 3:8

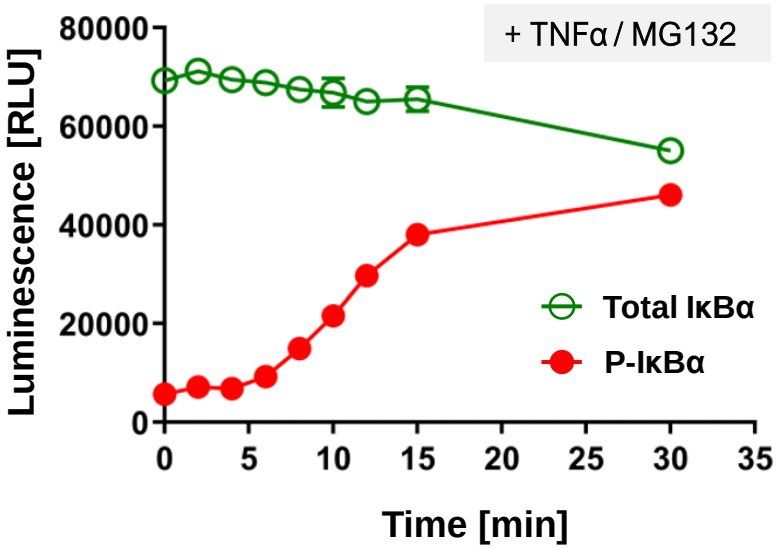
OBSERVATION

- IκBα phosphorylation at Ser32 (pS32)
- Immediately followed by rapid degradation

Signaling Pathway and Kinase Activity Analysis

Studying the NFκB Pathway





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OBSERVATION

- Decrease in IκBα degradation
- Accumulation of phosphorylated IκBα

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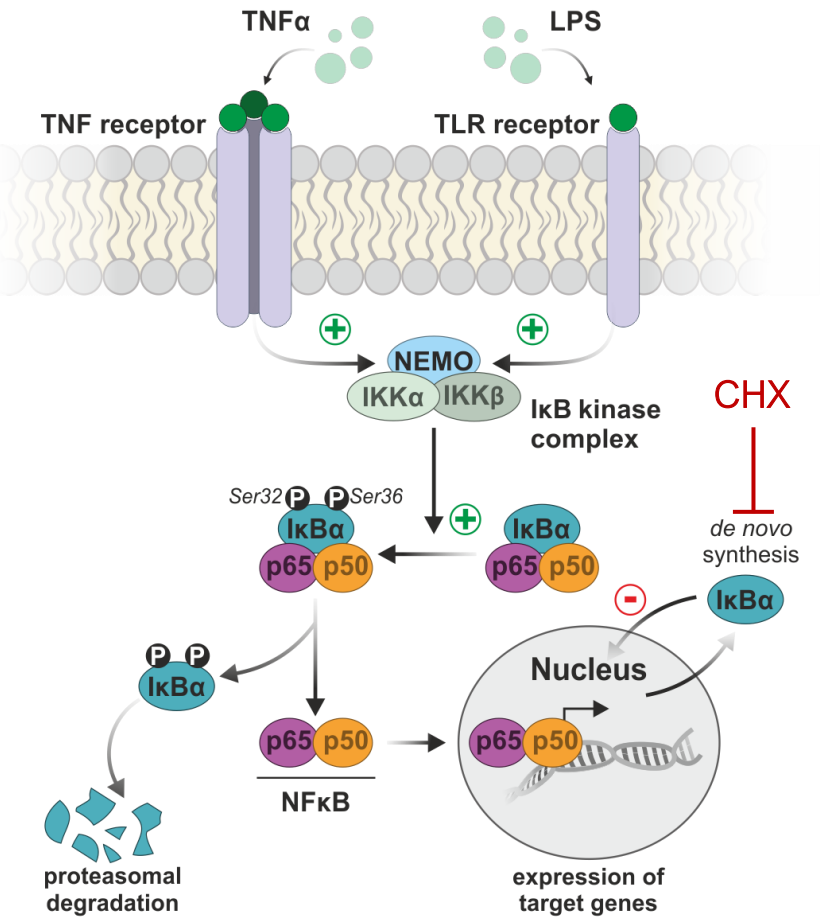

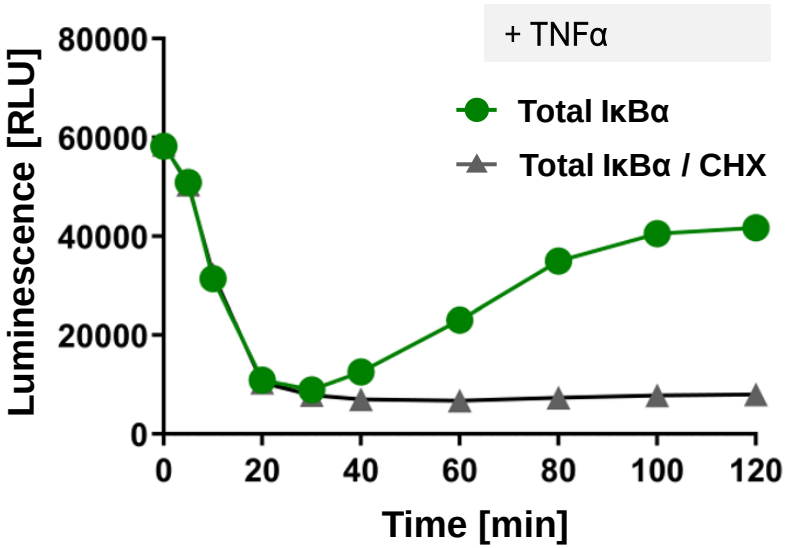

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OBSERVATION

- Cycloheximide (CHX) blocks NFκB-triggered *de novo* IκBα biosynthesis

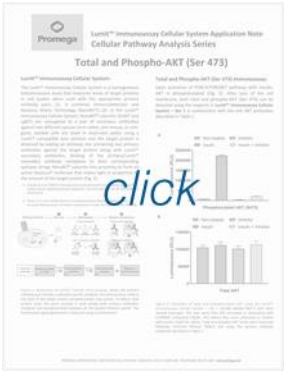
Lumit™ Immunoassay Cellular Systems

A Universal Immunoassay to Study Cellular Signaling

Validated with >20 phospho- and total proteins using 8 cell types, suggesting this universal immunoassay can be adapted for any pathway **with the appropriate antibodies**

- IκBα (phosph-Ser32 and total protein)
- STAT3 (phospho-Tyr705 and total protein)
- BTK (phospho-Tyr223)
- Estrogen receptor (total protein)
- β-Catenin (total protein)
- CREB (phospho-Ser133)
- P38 MAPK (phospho-Thr180/182)
- NFκB p65 (phospho-Ser536 and total protein)
- AKT (phospho-Ser473, phospho-Thr308, and total protein)
- Retinoblastoma tumor suppressor protein (phospho-Ser807/811 and phospho-Ser780)
- S6 ribosomal protein (phospho-Ser235/236, phospho-Ser240/244)
- MEK1/2 (phospho-Ser217/221, phospho-Ser298)

Application Notes



Available on Promega website with information on antibodies used

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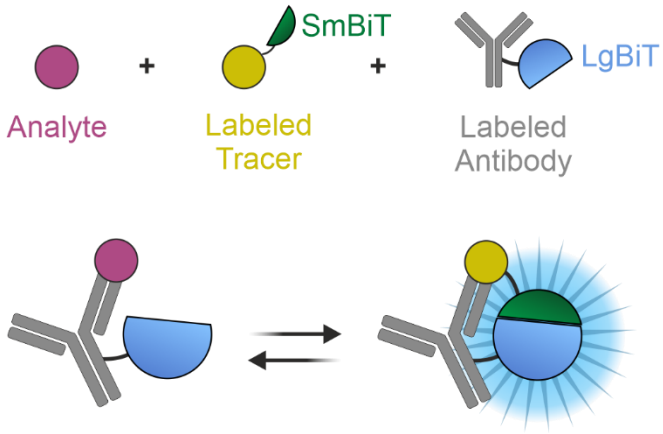

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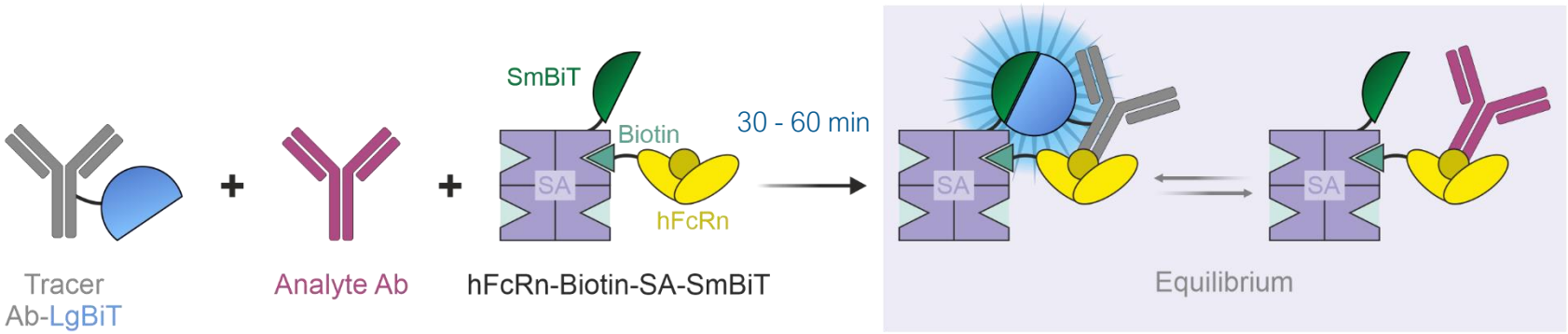
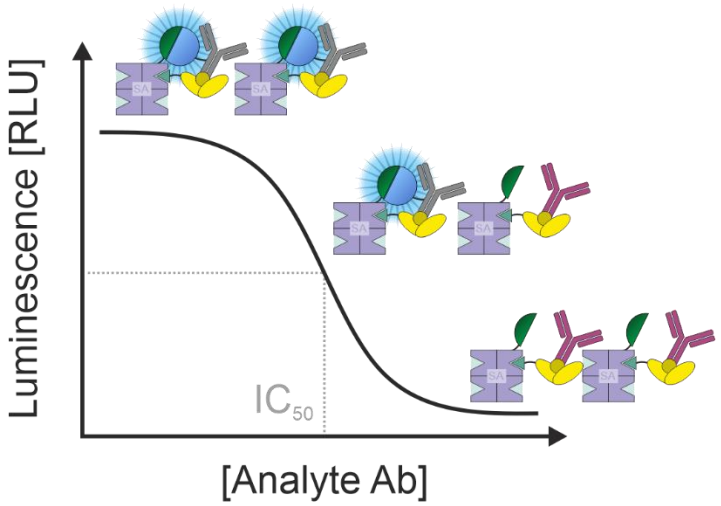
Lumit™ FcRn Binding Immunoassays

Neonatal Fc Receptor Binding Studies without Immobilization Artifacts



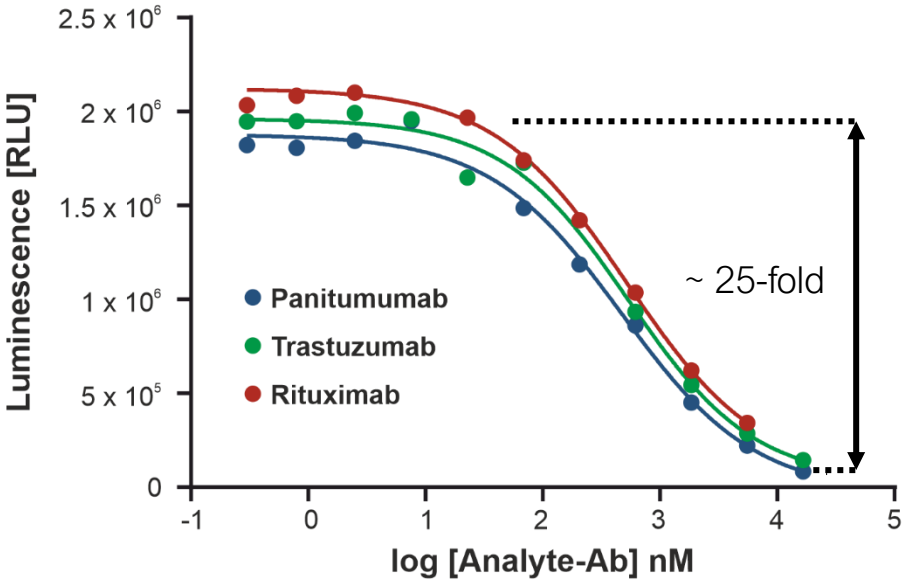
Facts

- Tracer: hIgG1 chemically labeled with LgBiT
- Biotinylated hFcRn bound to Streptavidin SmBiT
- Ab-LgBiT:hFcRn SmBiT yields luminescent signal
- Analyte antibodies that bind to hFcRn cause displacement of tracer, i.e. dose dependent decrease in luminescence



Lumit™ FcRn Binding Immunoassays

Analysis of Different Approved Therapeutic Antibodies



Antibody	IC_{50} (nM)
Panitumumab	458
Trastuzumab	553
Rituximab	471

OBSERVATION

- Relatively similar IC_{50} values determined for different therapeutic antibodies as expected
- Good assay window for hIgG:hFcRn binding studies

Summary – Lumit™ Immunoassays

A Bioluminescent Immunodetection Platform based on NanoBiT® Technology

THANK YOU!

Features of Lumit™ Immunoassays

- Homogenous “add-and read” format can be performed directly on cells
- Fast, 30 to 120 minutes total assay time
- Large dynamic ranges, i.e. no need for sample dilutions
- Use standard plate-reading luminometer
- Scalable to different plate formats (96- and 384-well)
- Amenable to high throughput applications
- Platform includes ready-to-use kits for specific targets as well as reagents to build novel detection assays

- For questions please contact:
  erik.bonke@promega.com

- Contact your local sales representative for products & sales relevant information:
  www.promega.com/c/local-sales/sales-contacts/