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For the Better

HUMAN HEALTH | ENVIRONMENTAL HEALTH

Cellular and Biochemical Assays Utilizing PerkinElmer Technologies: Applications for RNAi Research

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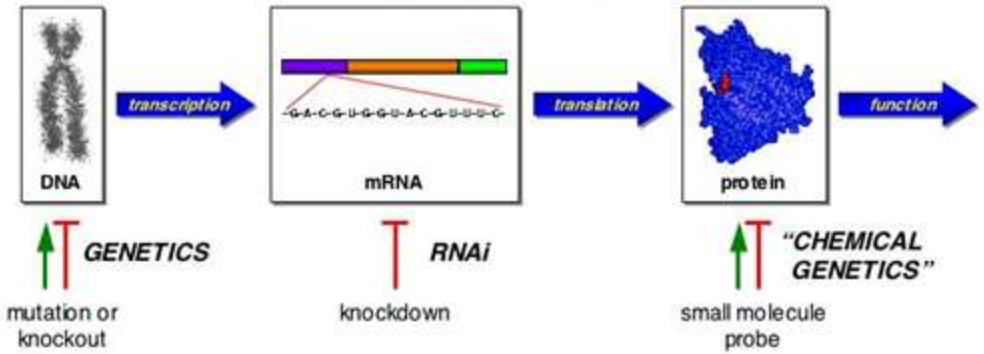
Presentation Outline



End Point Assay
- Phenotypic
- Specific

1. What is Alpha Technology
   - ✓ AlphaLISA – "Mix and Read" alternative to ELISA
   - ✓ SureFire Cellular Kinase Assays
   - ✓ Toolbox Beads (interaction, kinase, protease, etc)
   - ✓ Epigenetics (post-translation modification)

2. What is Time-Resolved Fluorescence (TRF)
   - ✓ Delfia
     - ✓ Immunoassays
     - ✓ In-The-Well" Cellular Analysis
     - ✓ Proliferation (pulse-chase DNA Synthesis)
     - ✓ Cytotoxicity

Multimode Detection Instrumentation for Assay Quantitation

EnSpire

- **Label Free – New Feature**
- **Alpha Technology** - Fast
- Absorbance
- Luminescence - Ultra-sensitive
- Fluorescence Intensity
- Filter or Monochromator (Quad)
- Time-Resolved Fluorescence
- Optional 21 CFR Part 11 compliance

Envision

- **Alpha Technology** – Std or HTS
- Absorbance
- Luminescence – Std or Ultra-sensitive
- **Time-Resolved Fluorescence**
- Fluorescence Polarization
- Fluorescence Intensity
- Filter or Monochromator (Quad)
- Optional 21 CFR Part 11 compliance

Assay Selection Key to Study Signal Transduction in Biological Model

Antibody Effectors or Inhibitors

Ligand-Receptor Interaction

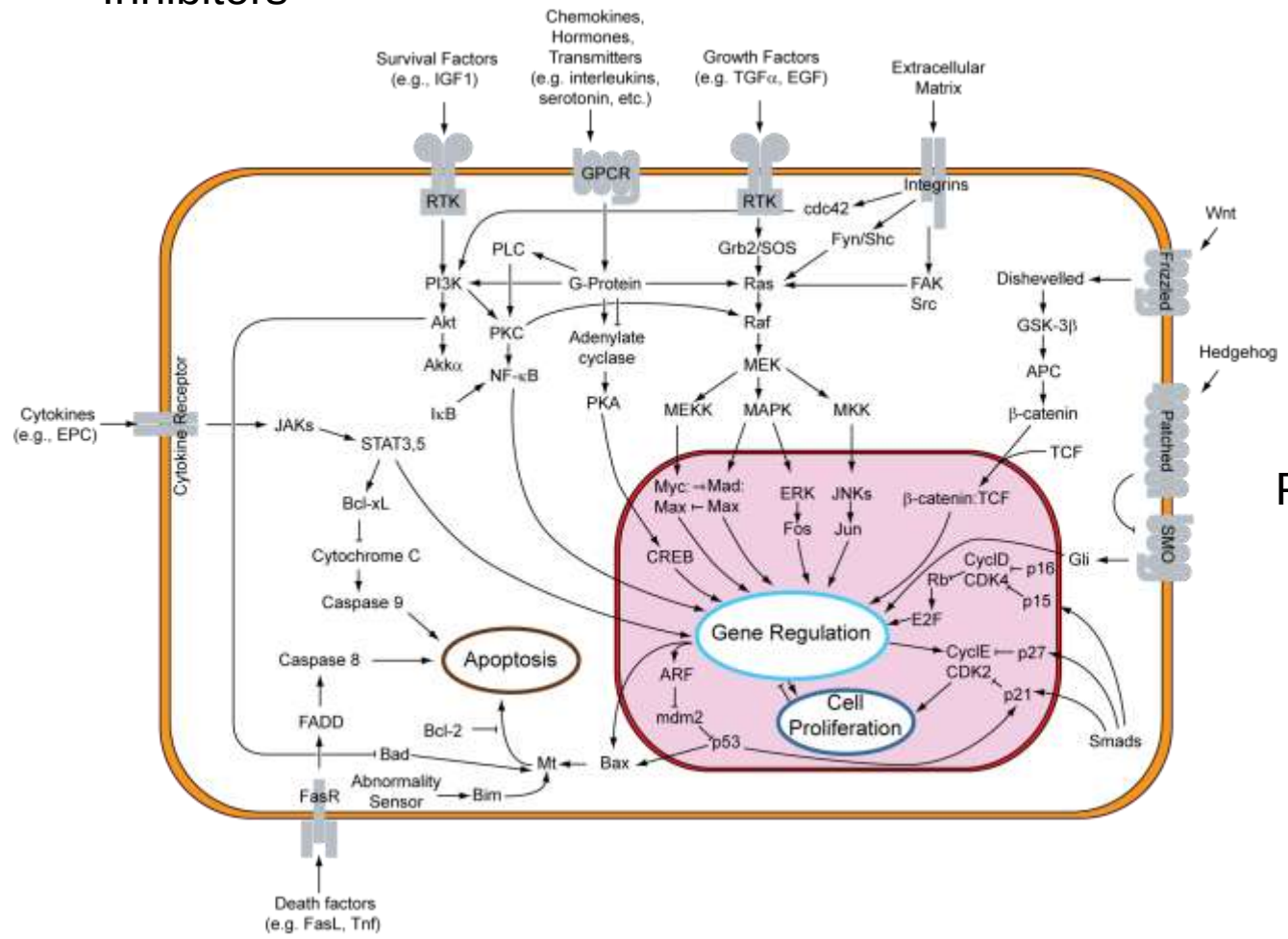
Biomarkers Secreted Proteins

Membrane Integrity ADCC

Signaling Pathways Phospho-Proteins Protein-Protein Interactions

DNA Synthesis Gene Activation

Epigenetic Activity Histone Modification



Signal Transduction Research Needs Assay Tools to Advance Discovery

Early Biomarker Detection by Radioimmunoassay (RIA)

Radiolabeled Insulin and primary antibody

Serum Insulin

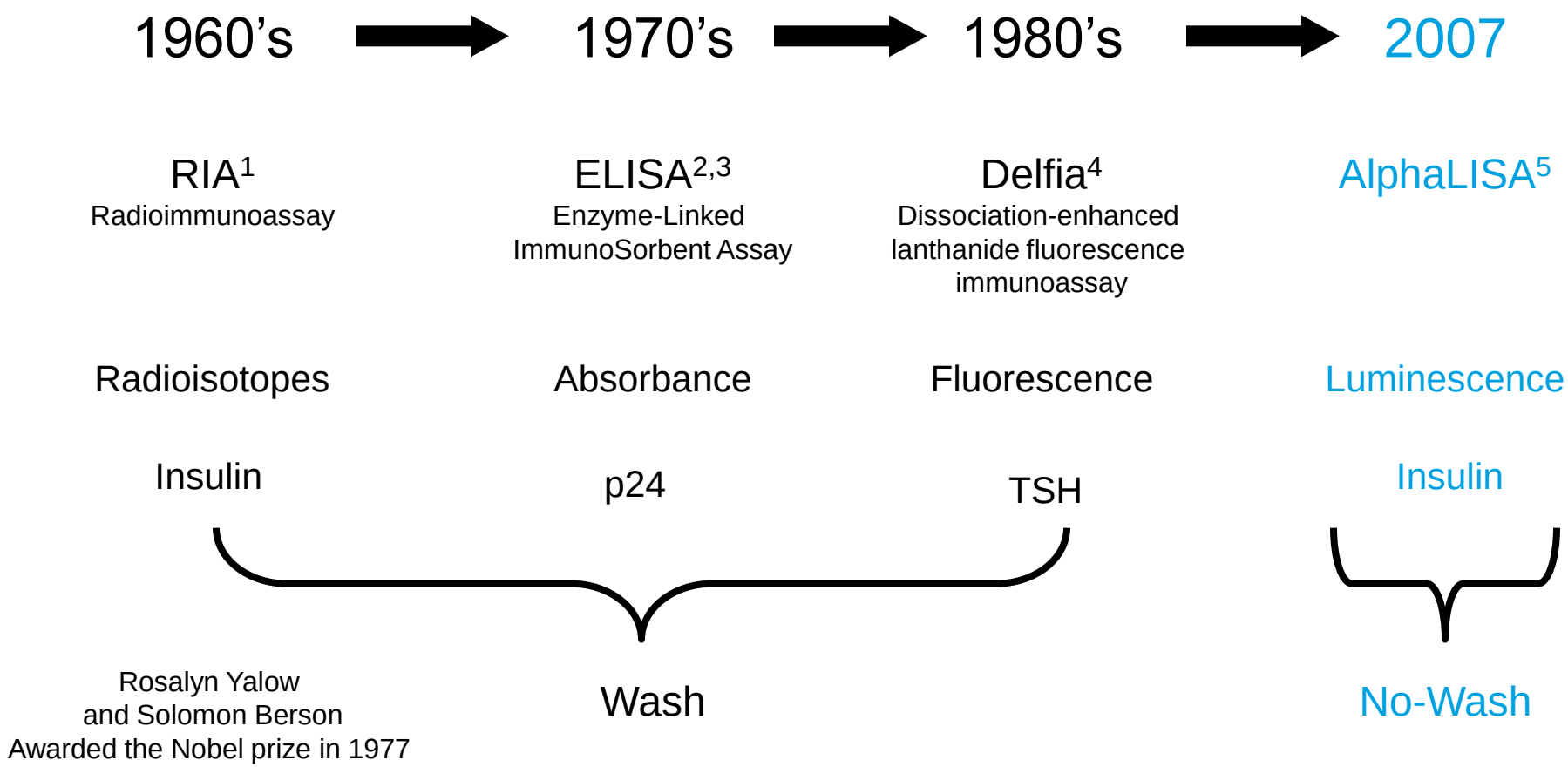
Serum Insulin competes with radiolabeled Insulin for antibody binding sites, forming an equilibrium

Capture on a Solid Surface Unbound insulin is removed

- The original immunoassay
- Developed in the late 1950's for insulin
- Rosalyn Yalow won the 1977 Nobel prize for her work

Measurement with PerkinElmer Ultima Gold and TriCarb

Evolution of Immunoassay Technologies for Biomarker Analysis



1. Yalow R and Berson S (1960). "Immunoassay of endogenous plasma insulin in man". J. Clin. Invest. 39: 1157-75
2. Engvall E, Perlman P (1971). "Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G". Immunochemistry 8 (9): 871-4
3. Van Weemen BK, Schuurs AH (1971). "Immunoassay using antigen-enzyme conjugates.". FEBS Letters 15 (3): 232-6
4. Meurman OH, Hemmilä IA, Lövgren TN, Halonen PE. (1982). " Time-resolved fluoroimmunoassay: a new test for rubella antibodies". J Clin Microbiol. 16(5):920-5.
5. Poulsen F. and Jensen KB (2007). " A Luminescent Oxygen Channeling Immunoassay for the Determination of Insulin in Human Plasma". J Biomol Screen. 12 (2):240-7

The origin of AlphaScreen/AlphaLISA

Ullman et al. : Bead-based homogenous detection of analytes

LOCI technology

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5426–5430, June 1994
Biochemistry

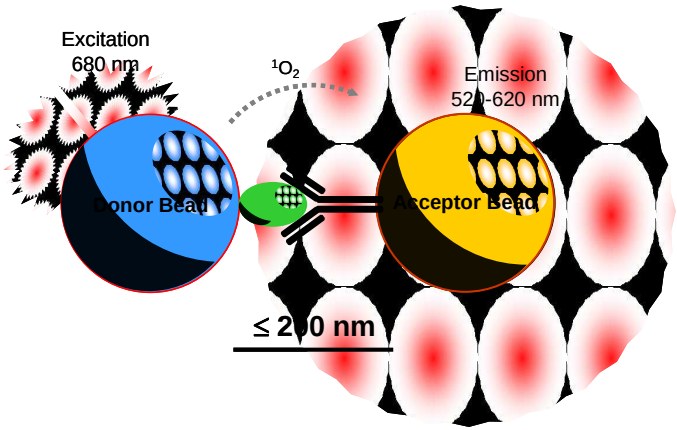
Luminescent oxygen channeling immunoassay: Measurement of particle binding kinetics by chemiluminescence

(homogeneous immunoassay/singlet oxygen/latex beads/antenna)

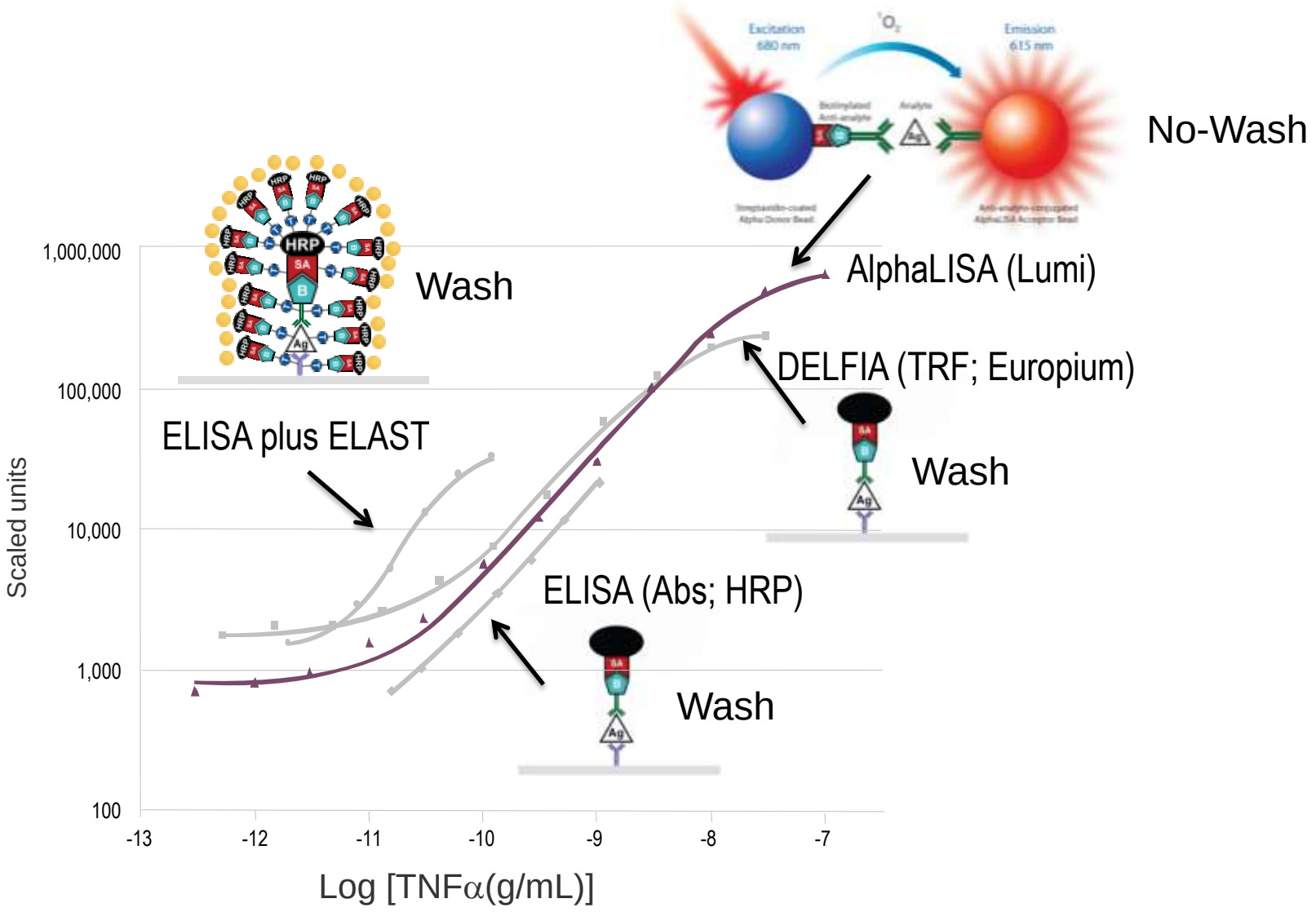
EDWIN F. ULLMAN*, HRAIR KIRAKOSSIAN, SHARAT SINGH, Z. PING WU, BENJAMIN R. IRVIN, JOHN S. PEASE, ARTHUR C. SWITCHENKO, JENNIFER D. IRVINE, ALAN DAFFORN, CARL N. SKOLD, AND DANIEL B. WAGNER

Research Department, Syva Company, Palo Alto, CA 94303

Communicated by Ronald Breslow, January 18, 1994

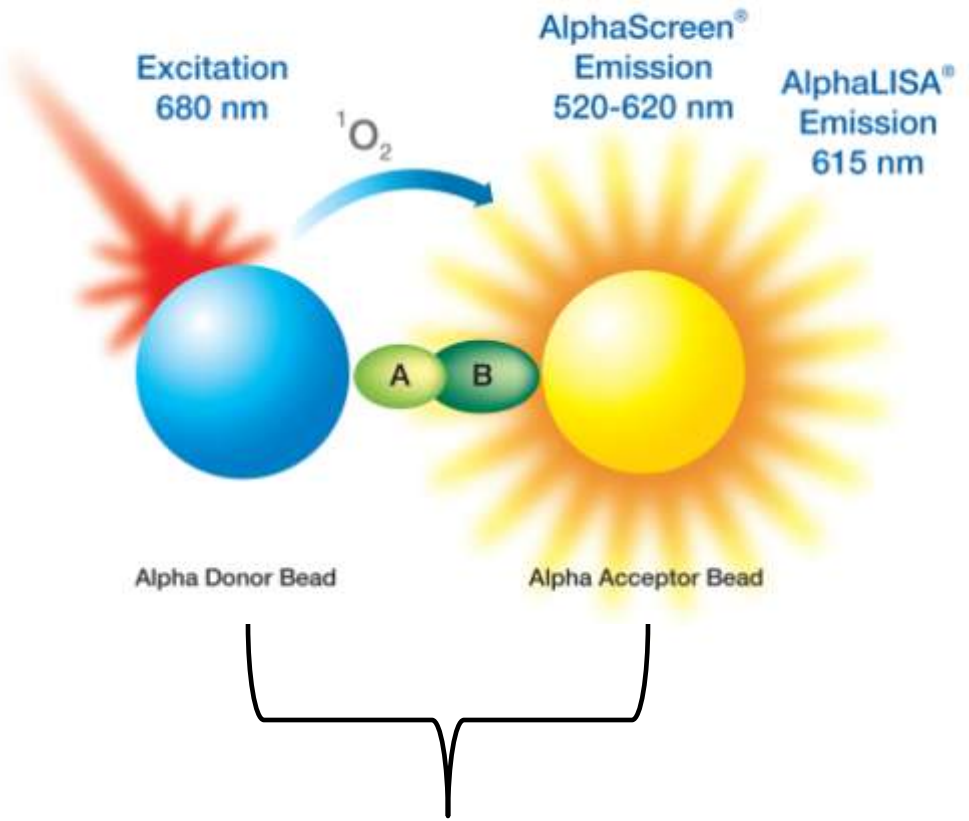


Comparison of Immunoassay-Related Technologies

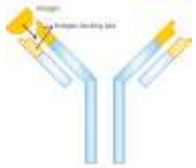


What is Alpha Technology – Proximity Detection

AMPLIFIED
LUMINESCENT
PROXIMITY
HOMOGENOUS
ASSAY



Bead Proximity Range ≤ 200 nm



Antibody
~7.5nm

Alpha Technology: Bead Characteristics

Donor beads

•**Antibody capture:**
- *Protein A*
- *Anti-rabbit IgG*
- *Anti-mouse IgG*

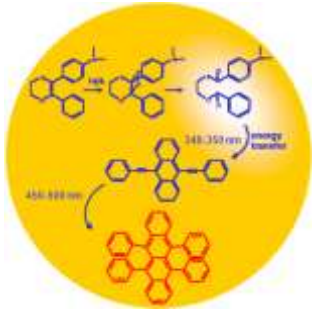
•**Fusion tag detection**:
- Streptavidin
- Nickel chelate
- Glutathione (GSH)
- *Anti-FLAG*
- *Strep-Tactin*

Unconjugated

$O_2 \xrightarrow{680\ nm} {}^1\Delta_g O_2$

AlphaScreen Acceptor Toolbox Beads

Unconjugated



Rubrene: **570 nm**

Donor-Acceptor Kits
- Ni-Chelate
- Glutathione
- Anti-species
- Anti-DIG
- Anti-FITC
- Protein A or G

AlphaLISA Acceptor Toolbox beads

Unconjugated

Eu: **615 nm**

•**Antibody capture**:
- Protein A
- Protein G
- *Protein L*
- Anti-human IgG
- Anti-rabbit IgG
- Anti-mouse IgG
- *Anti-mouse IgM*
- Anti-rat IgG
- Anti-goat IgG
- *Anti-sheep IgG*
- *Anti-chicken IgY*

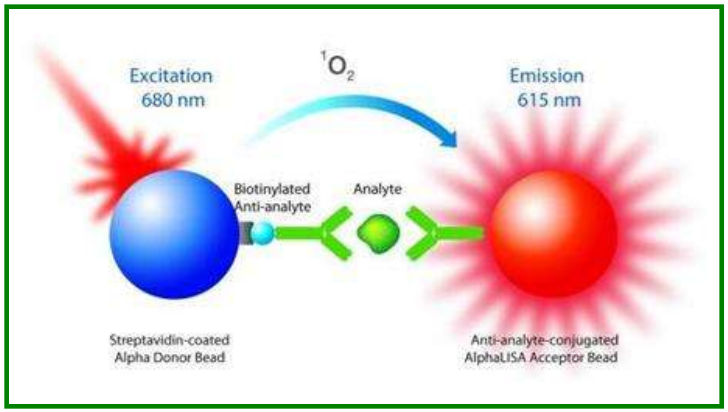
•**Fusion tag detection:**
- *Streptavidin*
- Nickel chelate
- Glutathione (GSH)
- Anti-FLAG
- Anti-GST
- Anti-c-myc
- Anti-DIG
- *Anti-FITC*
- *Anti-V5*
- *Anti-GFP*
- *Anti-MBP*
- *Strep-Tactin*

Beads

- Made of latex
- ~250 nm: very stable colloid suspension
- Hydrogel: dextran polymer
- Reactive aldehyde groups (raw beads)
- Heat stable (95°C)

Alpha Technology - AlphaLISA and AlphaScreen (LOCI)

- Same excitation
  - Donor beads
  - Singlet oxygen
- Different emission
  - AlphaLISA
    - Europium
  - AlphaScreen
    - Rubrene

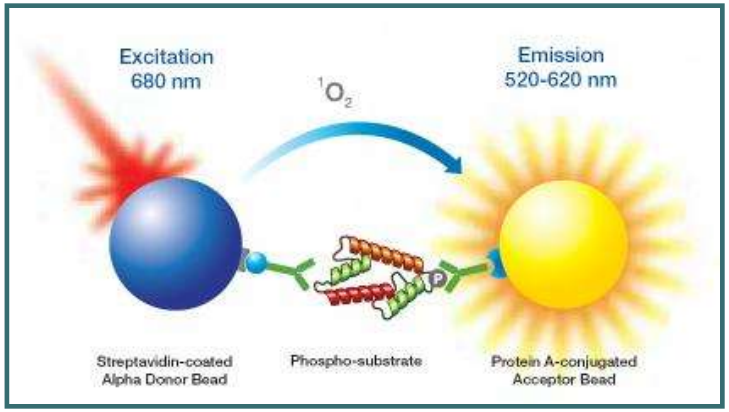


AlphaLISA

Biomarker

Protein-Protein

Epigenetic



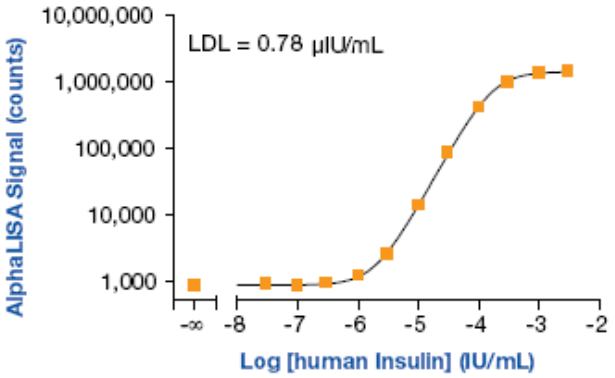
AlphaScreen

Phospho-Protein

AlphaLISA is very Flexible, Any Volume , Any Plate Density

Typical Results

Log scale



Specificity

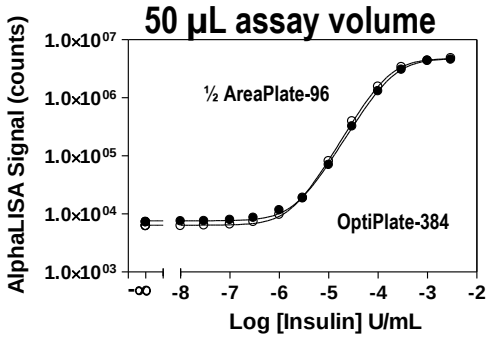
The AlphaLISA Insulin kit was tested against the following analytes:

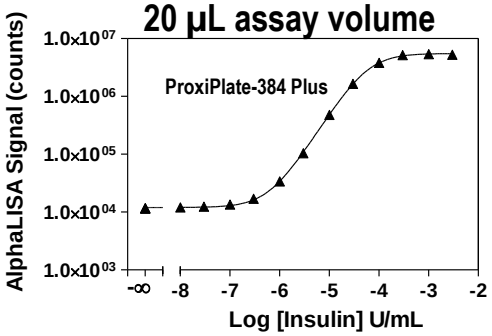
Analyte	Cross-reactivity
Mouse Insulin	100%
Rat Insulin	100%
Bovine Insulin	87%
Porcine Insulin	97%

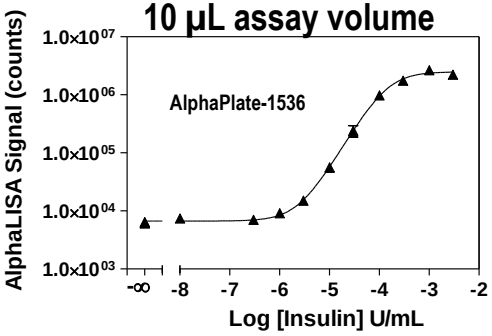
<u>In AlphaLISA buffer:</u>
LDL: 0.8 µIU/ml (26.6 pg/ml)
Dynamic range: 0.027 – 100 pg/ml

<u>In Analyte-depleted serum:</u>
LDL: 1.3 µIU/ml (43.2 pg/ml)
Dynamic range: 0.043 – 100 pg/ml

AlphaLISA Assay Miniaturization







Time-Resolved Fluorescence

Dissociation **E**nhanced **L**anthanide **F**luoro**I**mmuno**a**ssay

Time-Resolved Fluorescence (TRF)

TRF = Delfia

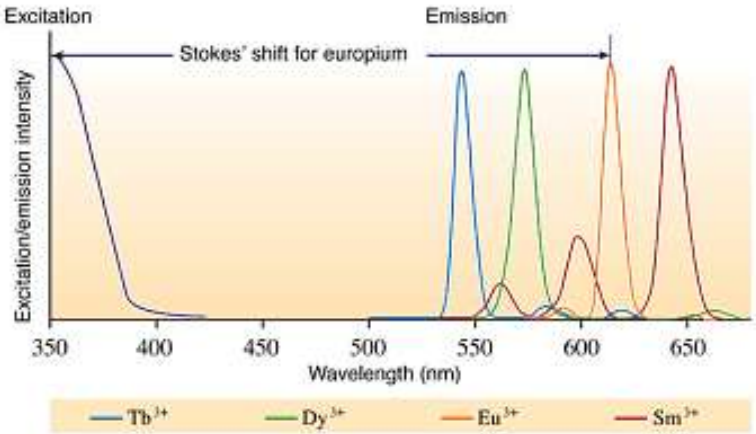
Sc														
Y														
La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Lanthanide as labels

- ✓Large Fluorescent yield

Lanthanide Chelates

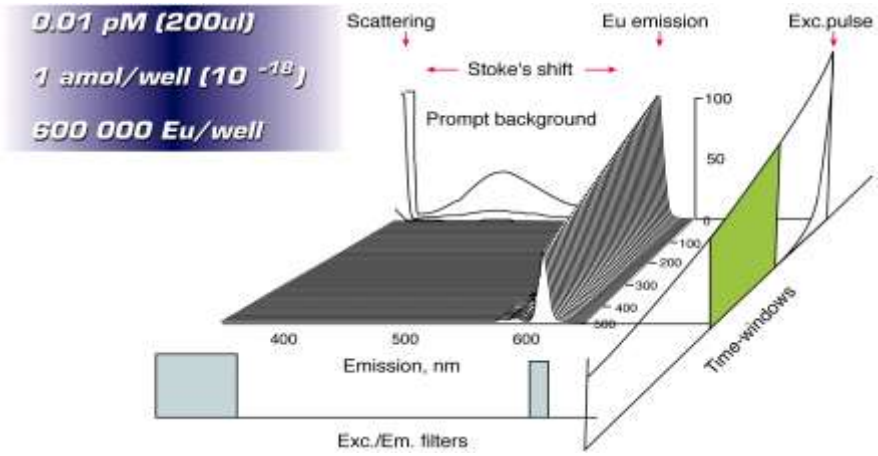
- ✓Chelate Aids in light collection
- ✓Allows conjugation to macromolecules



Lanthanides

- ✓ Large Stokes' shift
- ✓ Produces high S/B ratios

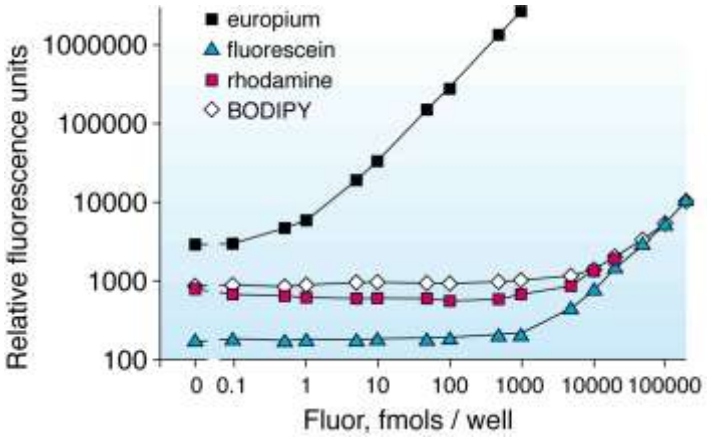
Time-Resolved Fluorescence (TRF)



Large Stokes Shift

Long signal decay times

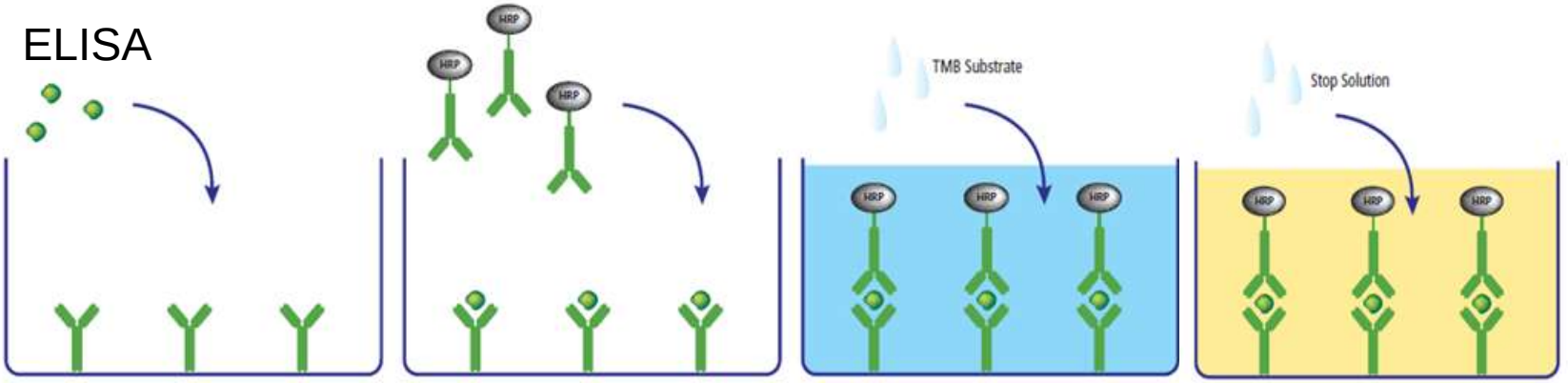
- ✓ Allows time-resolved fluorometry
- ✓ Minimizes interfering fluorescence from assay components



Lanthanides have Wider Dynamic Range and Enhanced Sensitivity Than Other Fluors

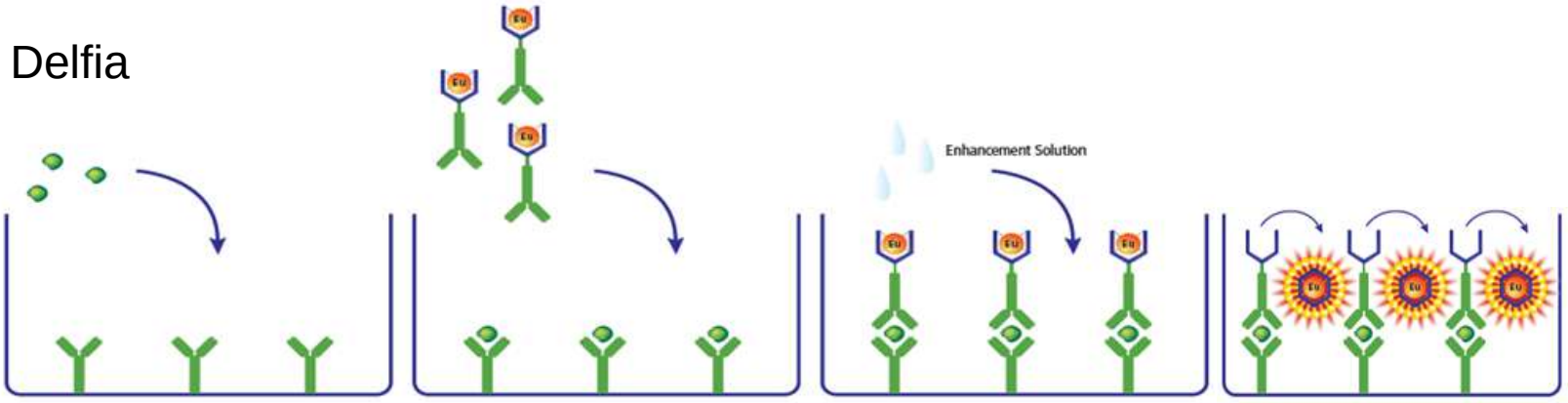
DELFIA and ELISA – Similar Assay Designs or Workflow

ELISA



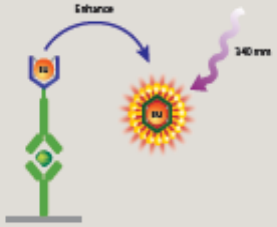
1. Capture analyte on antibody-coated microplate
2. Add HRP-labeled detection antibody to complete sandwich
3. Add substrate solution to yield colorimetric signal
4. Stop enzymatic reaction and read A450.

Delfia



1. Capture analyte on antibody-coated microplate
2. Add Europium-labeled detection antibody to complete sandwich
3. Add Enhancement solution to release Europium into solution
4. Measure time-resolved fluorescence

ELISA to Delfia Conversion

Comparing Assay Processes: ELISA Against PerkinElmer's DELFIA	
ELISA	**DELFIA**
	
Coat plate with capture antibody	
Add sample	
Incubate; wash	
Add HRP-labeled detection antibody	**Add Eu-labeled detection antibody**
Incubate; wash	
Add substrate; monitor closely	**Add Enhancement Solution**
Stop reaction	**(No stop reaction required)**
Read absorbance promptly	**Measure TRF**

DELFIA Tool Box

- Eu-anti-mouse IgG (AD0124)
- Eu-anti-rabbit IgG (AD0105)
- Eu-anti-human IgG (1244-330)

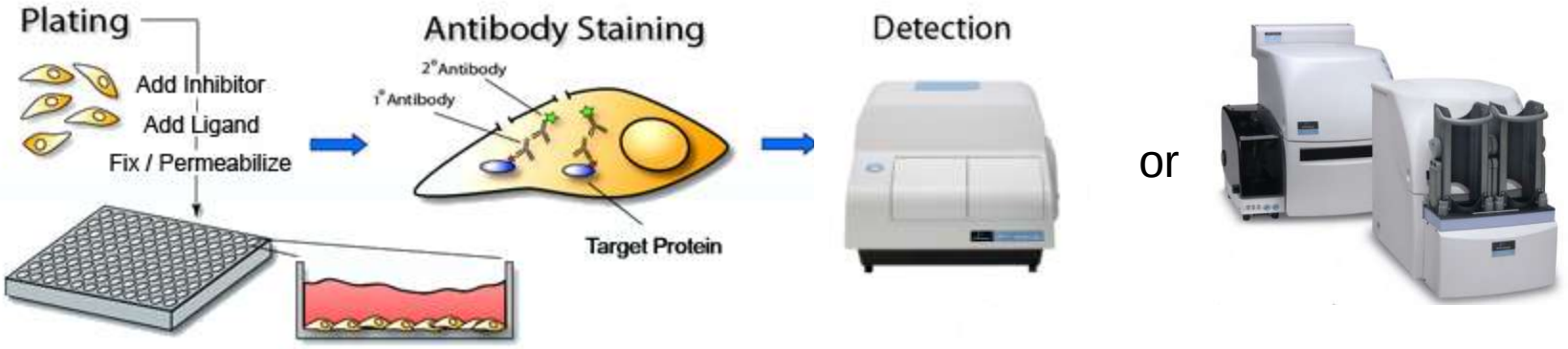
Indirect

- Eu-streptavidin (1244-360)
- Eu labeling kit (1244-302)

Direct

“In-the- Well” Cellular TRF (Delfia) Assay

Journal of Immunological Methods, Volume 291, Issues 1-2, August 2004, Pages 123-135
A cell-based time-resolved fluorescence assay for selection of antibody reagents for G protein-coupled receptor immunohistochemistry , Jui-Lan Su, et al Department of Gene Expression and Protein Biochemistry, GlaxoSmithKline Research and Development,



celTRF assay

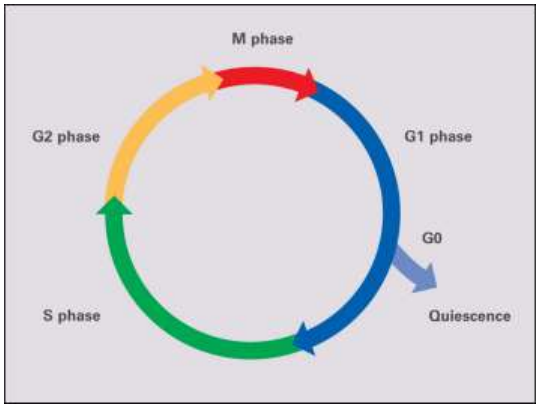
1. Treat Cells in 96 well plates
2. Remove culture media and **add fixative** for 20 min.
3. Wash with PBS plus 0.3% Triton X-100
4. **Permeabilization** for 15 min.
5. Blocking from 30 min to overnight
6. Add anti-target affinity-purified rabbit antibody for 1 hour
7. Wash 3× with PBST at 5 min per wash.
8. Europium-labeled goat anti-rabbit IgG for 1 h.
9. Washed as above 5× prior
10. Add Enhancement Solution for 15 min
11. TRF (Delfia) signals were counted in multilabel counter .

Conclusion of Paper

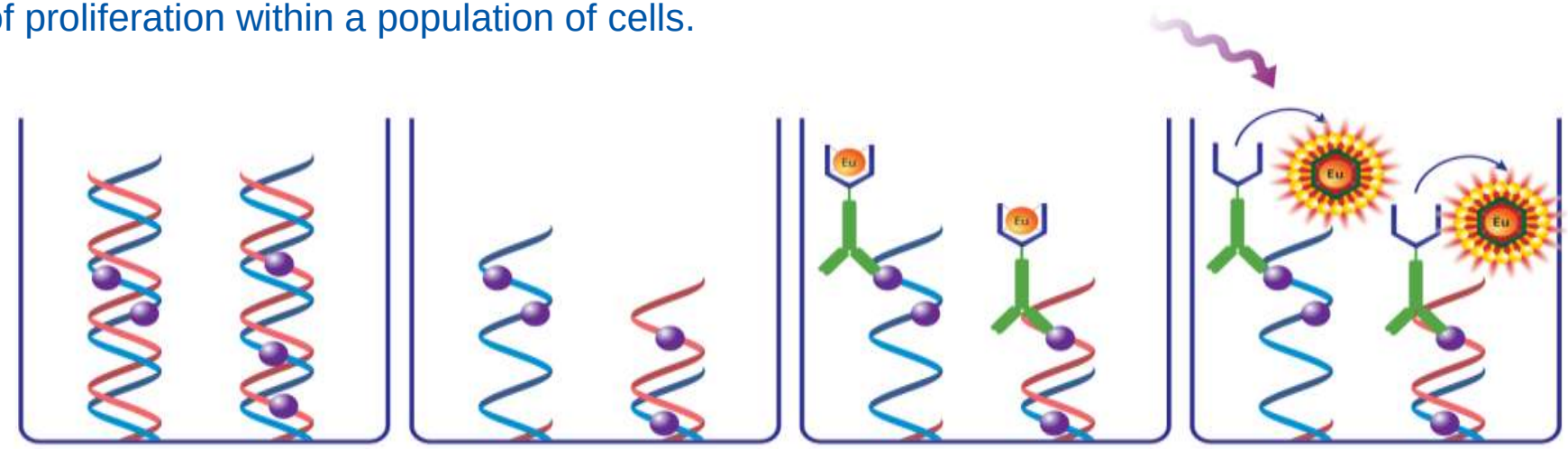
These results indicated that the antibody ranking based on the celTRF assay closely correlated with IHC results. In contrast, a high ELISA titer did not always correlate with activity in IHC localization

The DELFIA Cell Proliferation Kit

Assay Principle: During mitosis, DNA is replicated during S-phase of the cell cycle. DNA synthesis is achieved by the replication machinery through incorporation of free nucleotides into the growing DNA strands. BrdU is an analog of thymidine which will also be naturally incorporated into the DNA if added to proliferating cells.



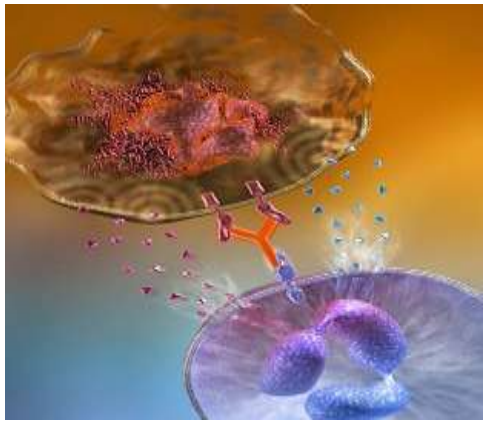
By culturing cells in the presence of BrdU and subsequently detecting the level of BrdU incorporation, one can gain a measure of proliferation within a population of cells.

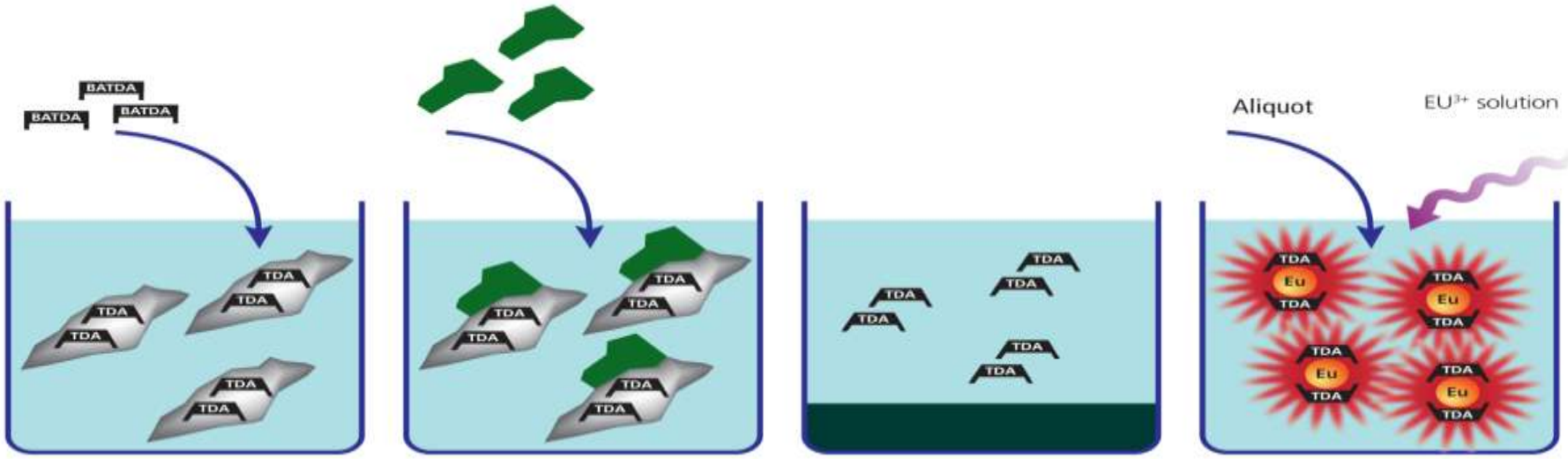


1. Label cells with BrdU.
2. Denature DNA to expose BrdU label.
3. Add Europium-labeled anti-BrdU detection antibody.
4. Add Inducer solution and measure TRF.

A DELFIA® TRF cell-mediated cytotoxicity assay

Assay Principle: Cell lysis is monitored through detection of a fluorescence enhancing ligand (BATDA) that is loaded into target cells. BATDA is a hydrophobic ligand that easily passes through the plasma membrane. Upon entry into the cell, BATDA is hydrolyzed by cellular esterases to the hydrophilic molecule TDA, which cannot pass through the plasma membrane. Thus, the levels of cytolysis can be tracked by the amounts of TDA released into solution.





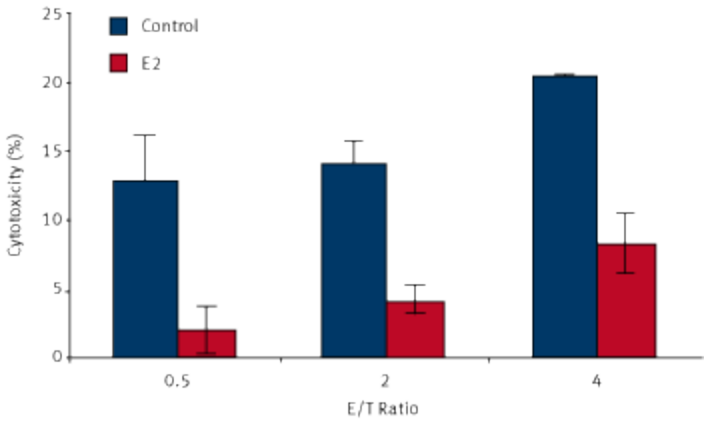
1. Add BATDA ligand to cells; BATDA converted to TDA by cellular esterases.

2. Add effector cells cells; cell-mediated lysis will release TDA into solution.

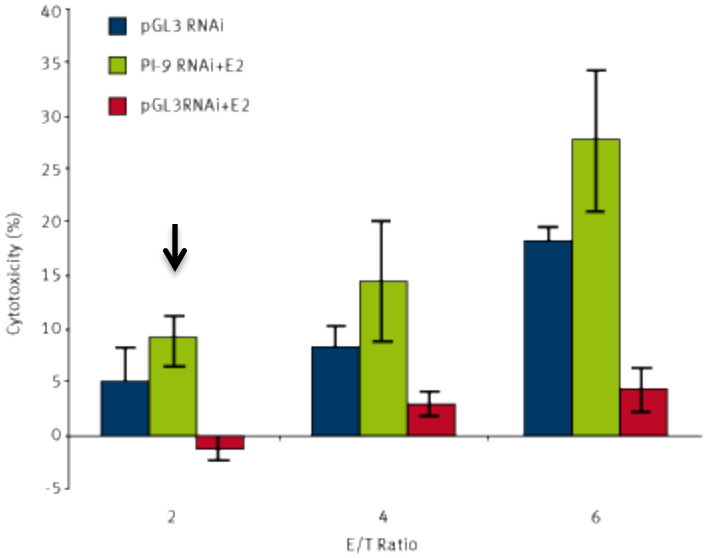
3. Centrifuge cellular debris; TDA ligand remains in supernatant.

4. Aliquot supernatant into Europium solution; TDA forms fluorescent complex with Europium, EuTDA. Detect with TRF.

Example of A DELFIA® TRF cell-mediated cytotoxicity assay



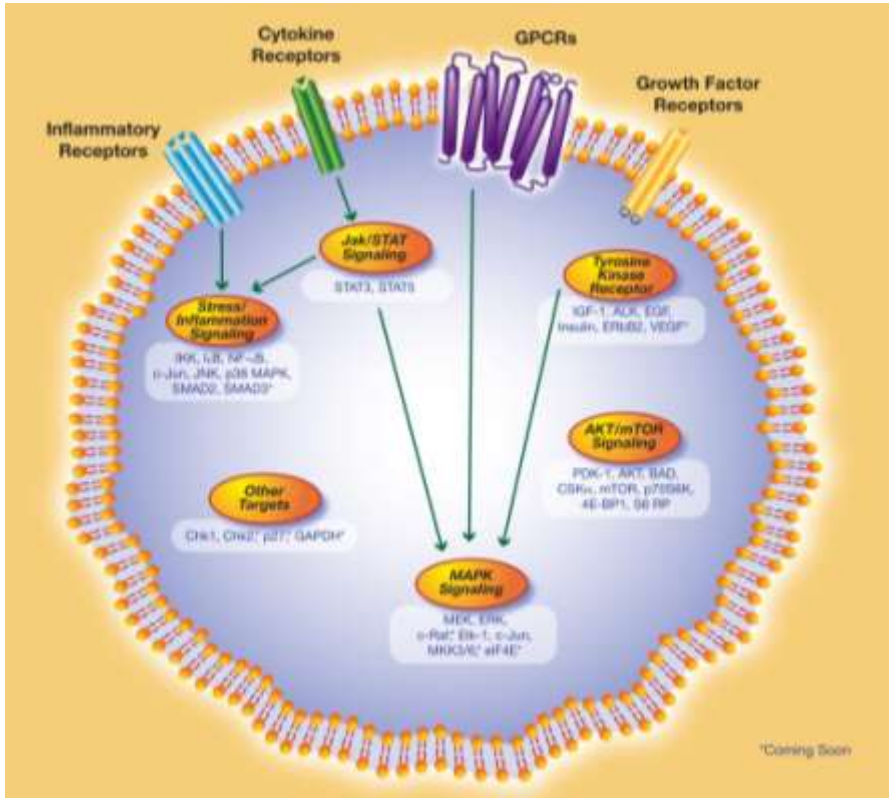
Estrogen (E2) protects MCF-7 cells against cytolysis induced by NK92 cells. MCF-7 target (T) cells were treated with ethanol vehicle (filled bars) or 10 nM E2 (open bars) for 24 hours, followed by incubation with NK92 effector (E) cells at different E/T ratios

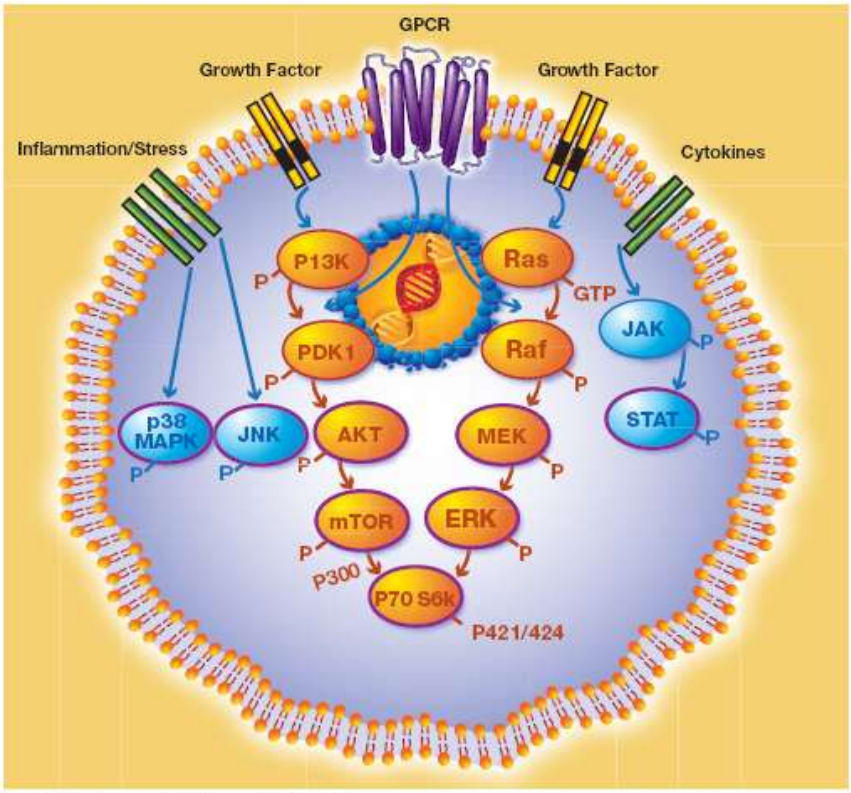


RNAi knockdown of PI-9 blocks estrogen protection against NK cell mediated cytotoxicity. MCF-7 cells were transfected with the control pGL3 luciferase siRNA, or with the PI-9 siRNA. After 24 hours, ethanol vehicle or estrogen (E2) was added and the cells were maintained for an additional 24 hours and incubated with the indicated ratios of effector NK92 cells to MCF-7 target cells and assayed for cytoxicity using the time resolved fluorescence assay.

Estrogen dramatically reduces cell death induced by NK92 natural killer cells

Pathway Mapping – Phospho-Proteins

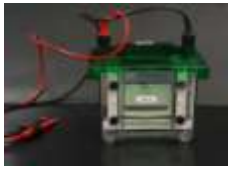




Available AlphaScreen *SureFire*® Kits are circled in purple.

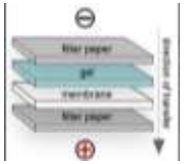
Western blotting workflow – Low sample throughput and qualitative


1 Sample Preparation



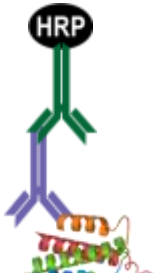

2 Gel Electophoresis

- Protein Markers

3 Transfer (electroblotting)

- Protran Nitrocellulose
- Polyscreen PVDF



4 Blocking & Immunodetection

- Secondary antibodies
- Blocking reagent

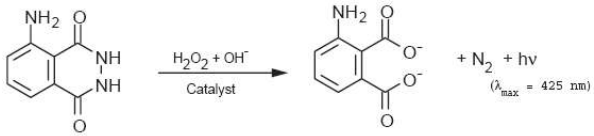


Figure 1: Oxidation of luminol with concomitant release of light.

5 Detection

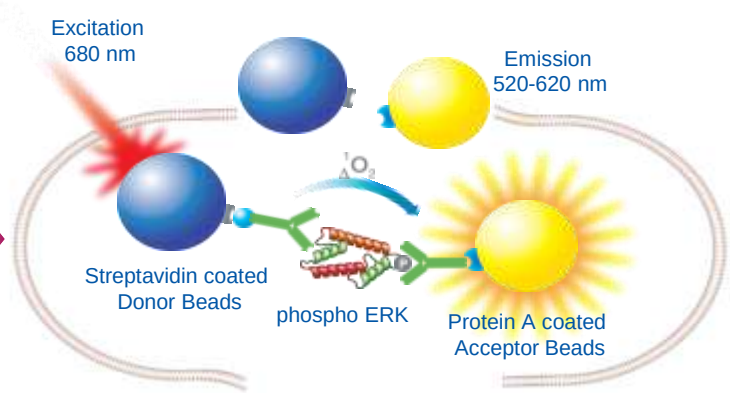
- Western Lightning Family
- Western BLAST


6 Imaging

- Kodak Film

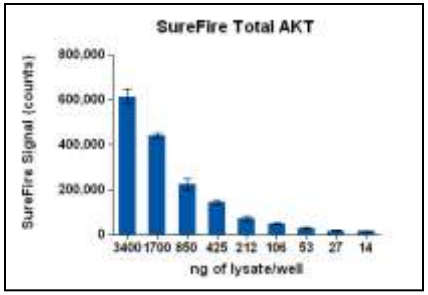
AlphaScreen SureFire - “Homogenous Cellular Western”

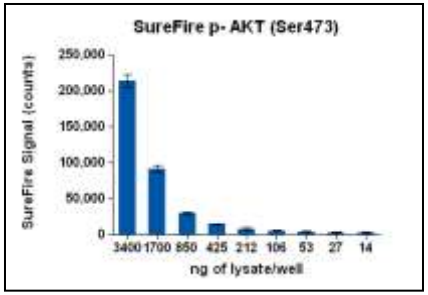
Cell Treatment

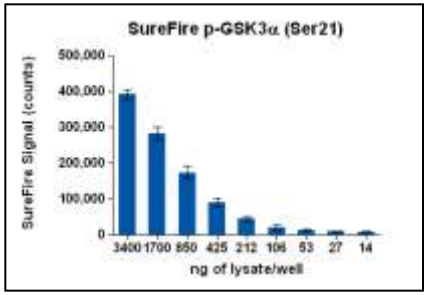


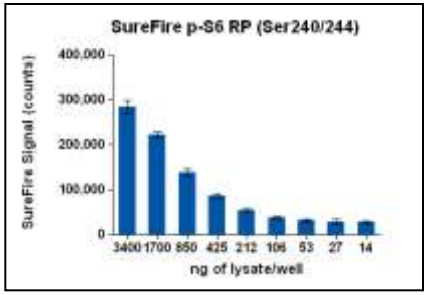
Cell Lysis

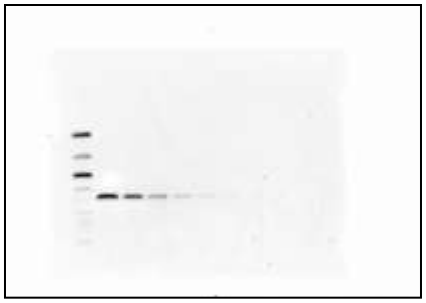
Phospho-Protein Detection

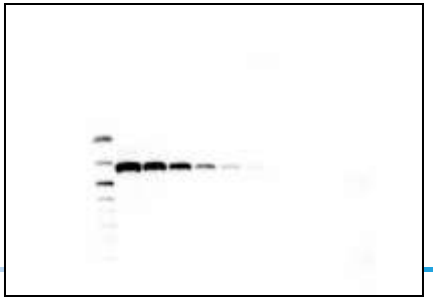




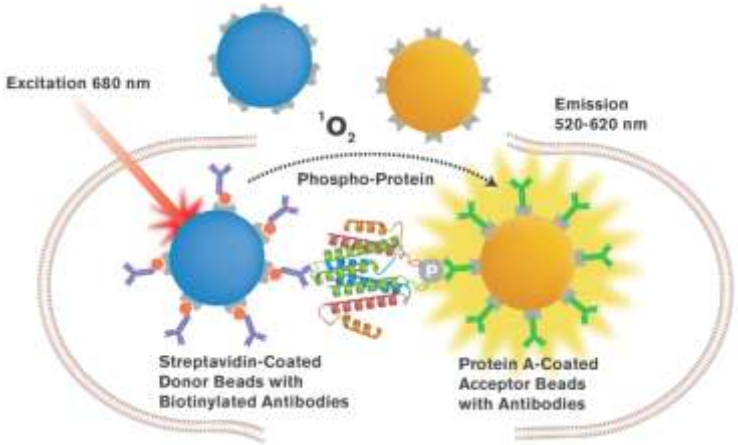


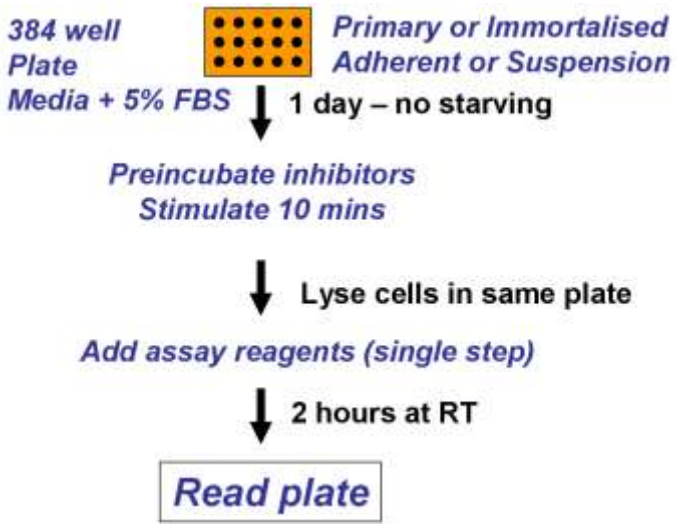




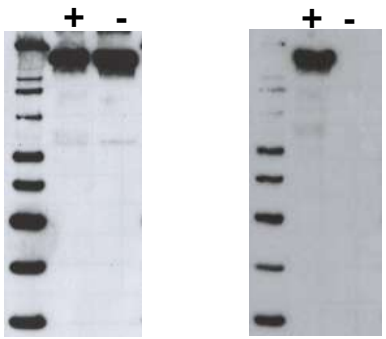


AlphaScreen SureFire Assay Principle and Workflow

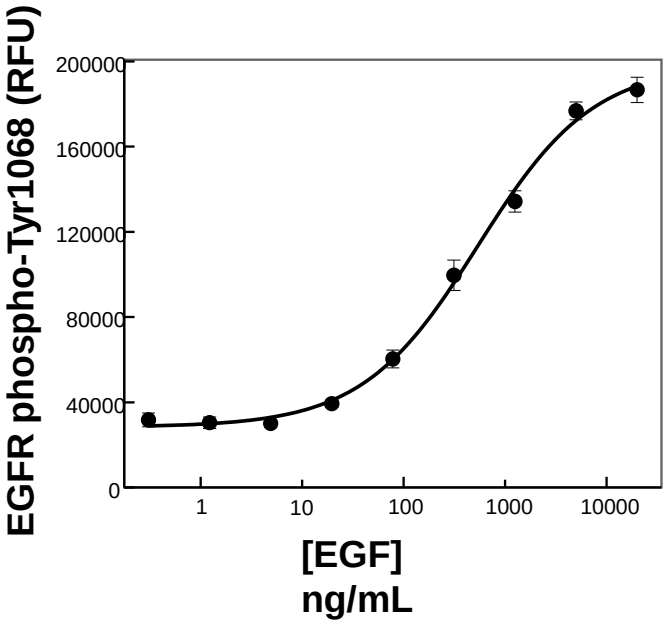




total EGFR EGFR p-Tyr1068.



A431 cells serum starved for 24 hours, then stimulated with EGF for 10 min



A431 cells in 96-well plates, serum starved for 3 hours, then stimulated with EGF for 10 min.

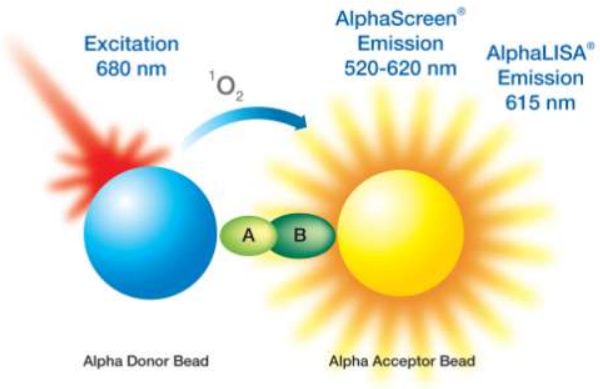
Surefire data is quantitative version of Western Blot

Alpha Toolbox Applications

Beads can be coated with antibodies or other binding molecules to develop virtually any assay type

- Protein-protein interactions
- Kinase Assays
- Protease Assays
- Epigenetic Assays
- Immunogenicity

Assay can be developed, as long as you can bring the beads together



Alpha Toolbox for Assay Development

Alpha Donor Beads

- Unconjugated

Antibody capture:
- *Protein A*
- *Anti-rabbit IgG*
- *Anti-mouse IgG*

Fusion tag detection:
- Streptavidin
- Nickel chelate
- Glutathione (GSH)
- *Anti-FLAG*
- *Strep-Tactin*

AlphaLISA Acceptor Beads

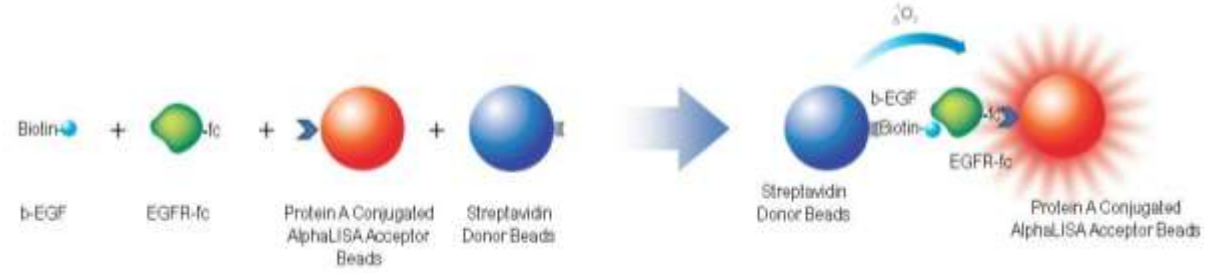
- Unconjugated

Antibody capture:
- Protein A
- Protein G
- *Protein L*
- Anti-human IgG
- Anti-rabbit IgG
- Anti-mouse IgG
- *Anti-mouse IgM*
- Anti-rat IgG
- Anti-goat IgG
- *Anti-sheep IgG*
- *Anti-chicken IgY*

Fusion tag detection:
- *Streptavidin*
- Nickel chelate
- Glutathione (GSH)
- Anti-FLAG
- Anti-GST
- Anti-c-myc
- Anti-DIG
- *Anti-FITC*
- *Anti-V5*
- *Anti-GFP*
- *Anti-MBP*
- *Strep-Tactin*

- protein-protein interactions
- enzymatic reactions
- detection of large particles

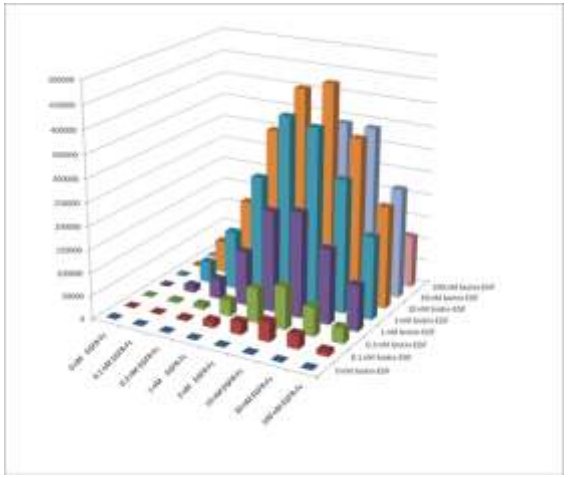
Alpha Protein-Protein assay design



Sample mapping for 96-well ½ AreaPlate

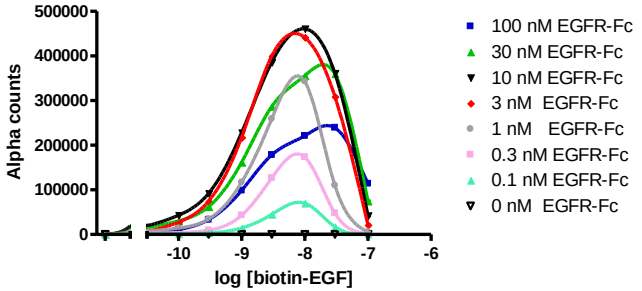
	100 nM EGFR-Fc 1	30 nM EGFR-Fc 2	10 nM EGFR-Fc 3	3 nM EGFR-Fc 4	1 nM EGFR-Fc 5	0.3 nM EGFR-Fc 6	0.1 nM EGFR-Fc 7	0 nM EGFR-Fc 8	(Empty) 9	(Empty) 10	(Empty) 11	(Empty) 12
A 100 nM biotin-EGF												
B 30 nM biotin-EGF												
C 10 nM biotin-EGF												
D 3 nM biotin-EGF												
E 1 nM biotin-EGF												
F 0.3 nM biotin-EGF												
G 0.1 nM biotin-EGF												
H 0 nM biotin-EGF												

Data for the cross-titration



Protein-protein interaction assay
1. Add 10 µL EGFR-Fc (final conc. 1 X 10^{-10} to 1 X 10^{-7} M)
2. Add 10 µL biotin-EGF (final conc. 1 X 10^{-10} to 1 X 10^{-7} M)
3. Incubate 60 min at room temperature
4. Add 10 µL Protein A Acceptor beads (final conc. 20 µg/mL)
5. Incubate 60 min at room temperature
6. Add 10 µL Streptavidin Donor beads (final conc. 20 µg/mL)
7. Incubate 30 min at room temperature
8. Read on an EnVision or EnSpire

Alpha protein-protein interaction Cross-titration



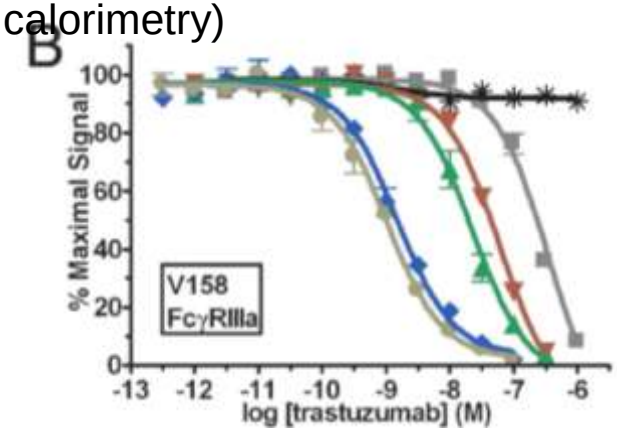
Engineered antibody Fc variants monitored by Alpha Binding Assay

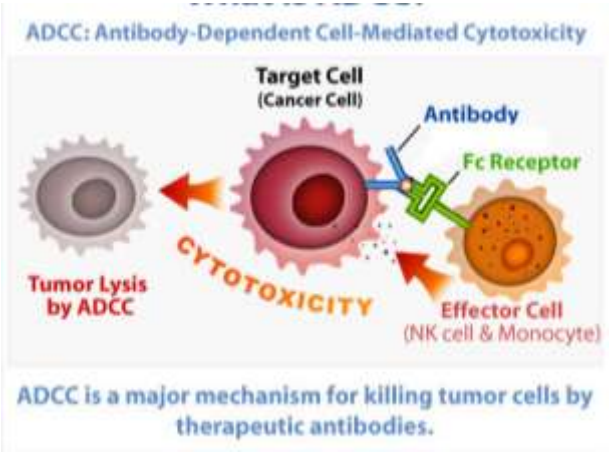
PNAS **March 14, 2006 vol. 103 no. 11 4005–4010**

Engineered antibody Fc variants with enhanced effector function

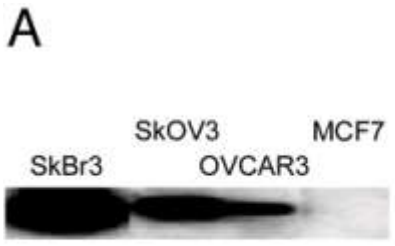
Greg A. Lazar*†, Wei Dang*, Sher Karki*, Omid Vafa*, Judy S. Peng, Linus Hyun, Cheryl Chan, Helen S. Chung,
Araz Eivazi, Sean C. Yoder, Jost Vielmetter, David F. Carmichael, Robert J. Hayes, and Bassil I. Dahiyat
Xencor, Inc., 111 West Lemon Avenue, Monrovia, CA 91016

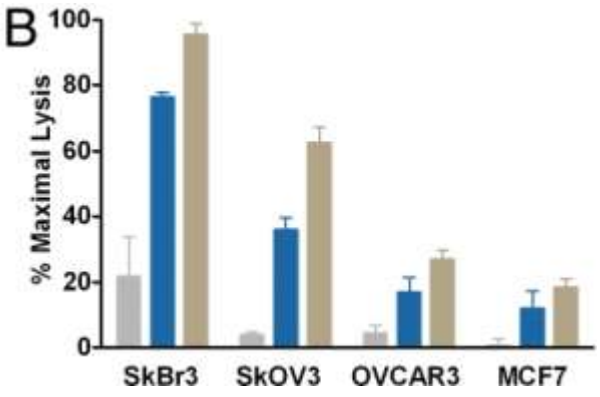
The WT Ab *Kd by Alpha agrees well with published data* (using SPR; or calorimetry)





Delfia Cell-based ADCC assay of trastuzumab Fc variants against cell lines expressing varying levels of Her2 receptor.





AlphaScreen predicted Ab potency correlates with Ab ADCC activity

Alpha - Interaction Assay

CELLULAR COFACTORS OF HIV INTEGRASE AS NOVEL ANTIVIRAL TARGETS
***Antivir Ther*. 2008; 13(Suppl. 3):P4 (abstract no. P2**
Z Debyser
KU Leuven Flanders, Belgium

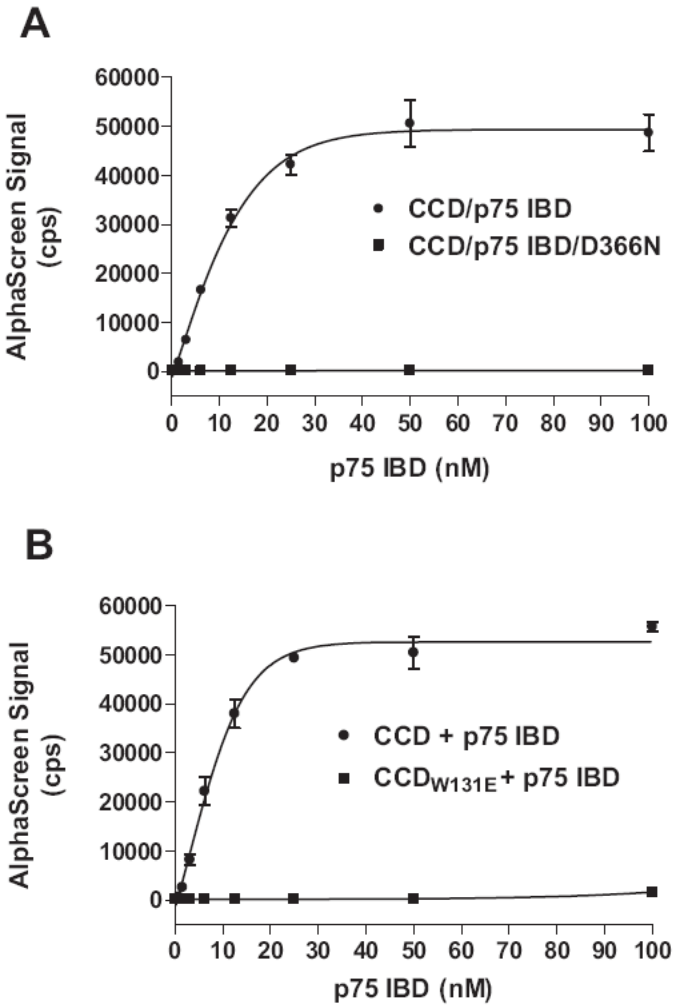
LEDGF/p75

1. binding partner of HIV-1 integrase
2. a chromosomal tether
3. role during HIV replication was independently confirmed by RNA interference (RNAi) knockdown

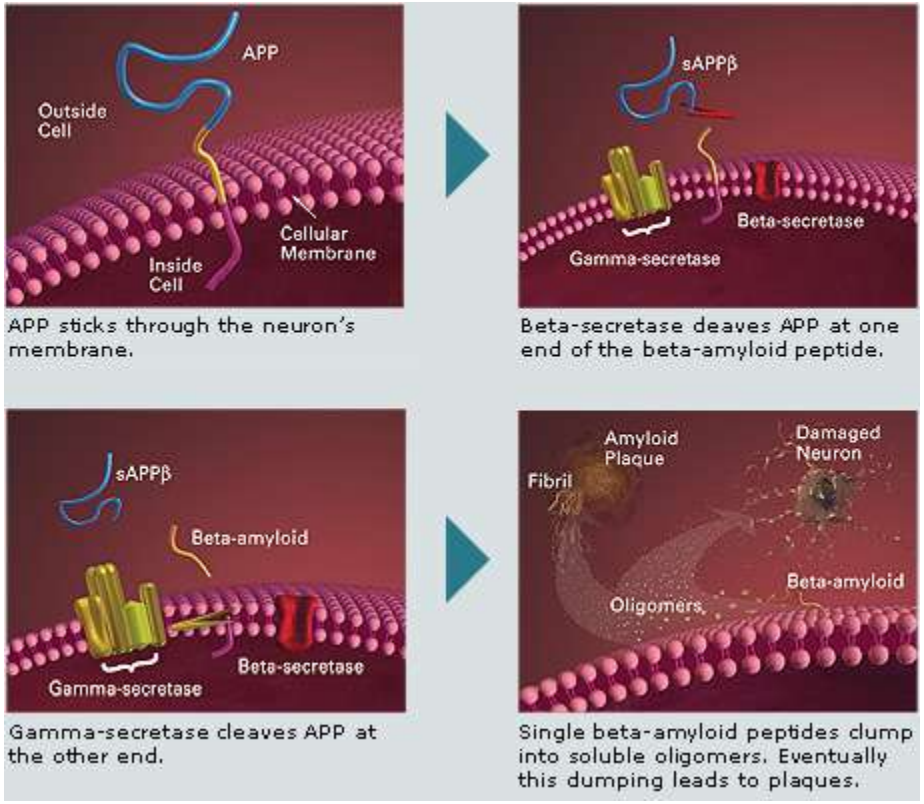
Table 1
LEDGF/p75 peptide AlphaScreen data for disruption of LEDGF/p75–IN complex formation

LEDGF/p75 peptide concentration	AlphaScreen counts	Percent inhibition[a] (%)
No peptide	247588.2	0
0.076 μM	250817	0
0.76 μM	291803.5	0
7.6 μM	169826	30.72
76 μM	77618	68.33
760 μM	18359	92.5
Control	2455.5	NA[b]

[a]Normalized for background signal.
[b]Not applicable.



Alzheimer's Disease



The role of the gamma-secretase complex in the amyloid-plaque formation pathway:

after beta-secretase cleaves APP,

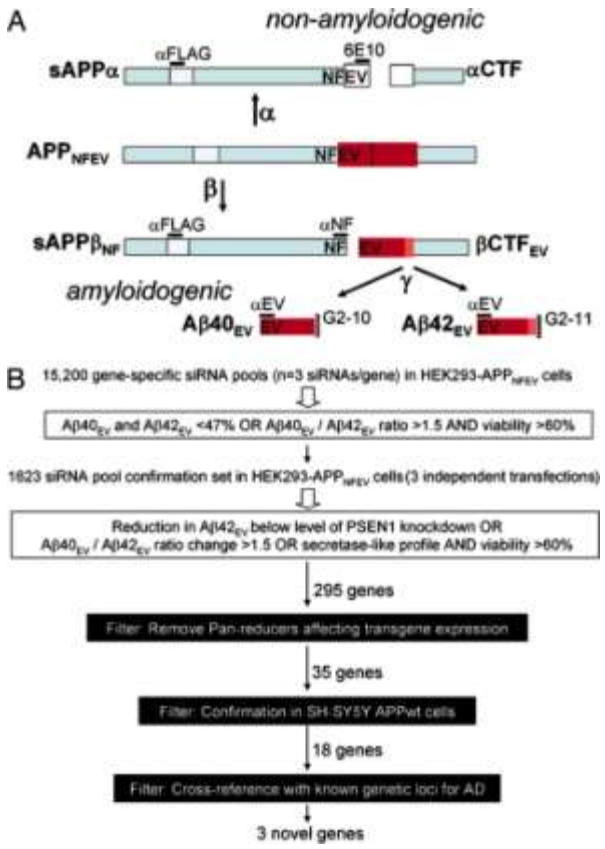
the beta segment may be cleaved again by gamma-secretase

acting inside the cell membrane, resulting in the formation of amyloid beta-peptides that exit the cell

and instigate the formation of amyloid plaques in the brain.

An siRNA screen for APP processing

LRRTM3 promotes processing of amyloid-precursor protein by BACE1 and is a positional candidate gene for late-onset Alzheimer's disease
Proc Natl Acad Sci U S A. 2006 Nov 21;103(47):17967-72



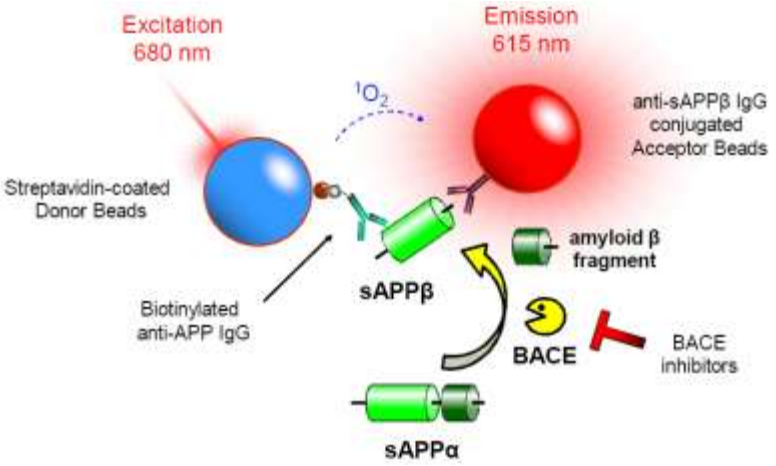
An siRNA screen for APP processing.

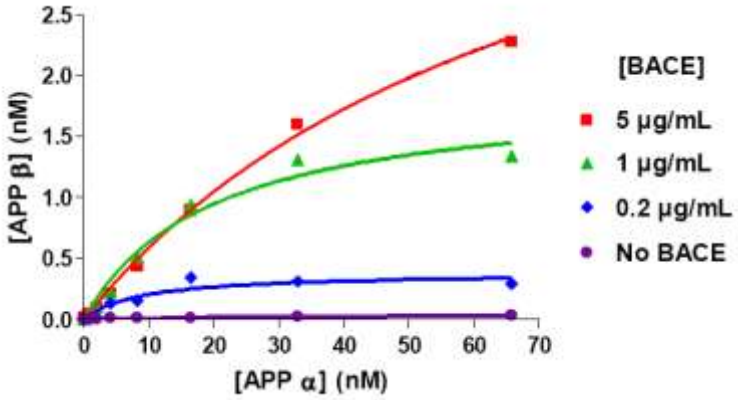
(A) APP processing by either α-secretase (nonamyloidogenic), generating sAPPα and the αCTF, or by BACE1 (amyloidogenic), generating sAPPβNF. The βCTFEV is a substrate for γ-secretase, producing the peptides Aβ40EV or Aβ42EV. Antibodies used are shown.

(B) Flow chart for siRNA screening and selection.

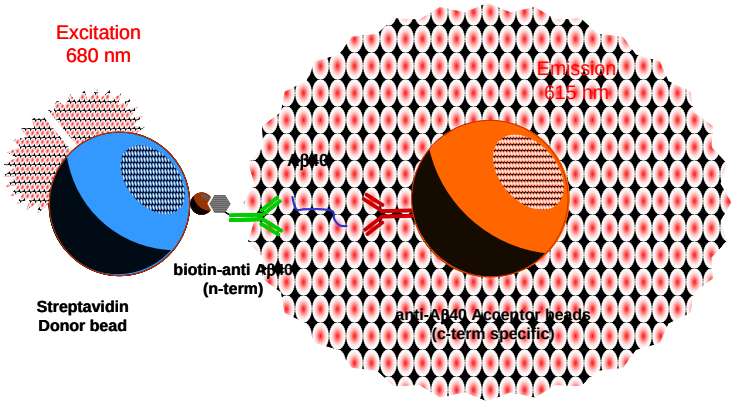
End Points

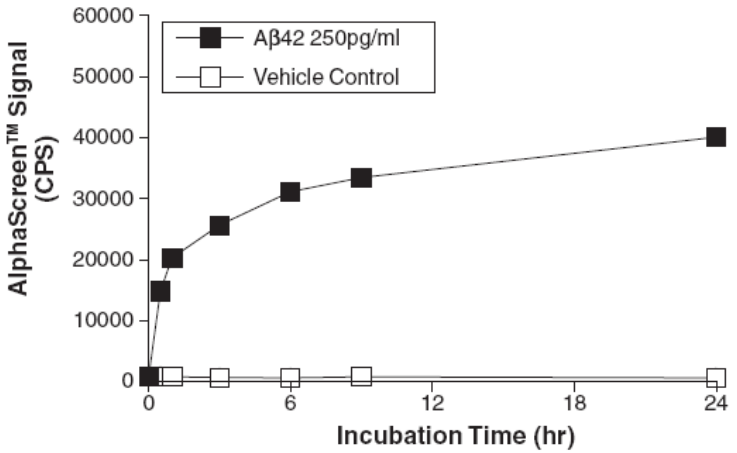
BACE Activity Assay





Aβ40 or 42 Assay





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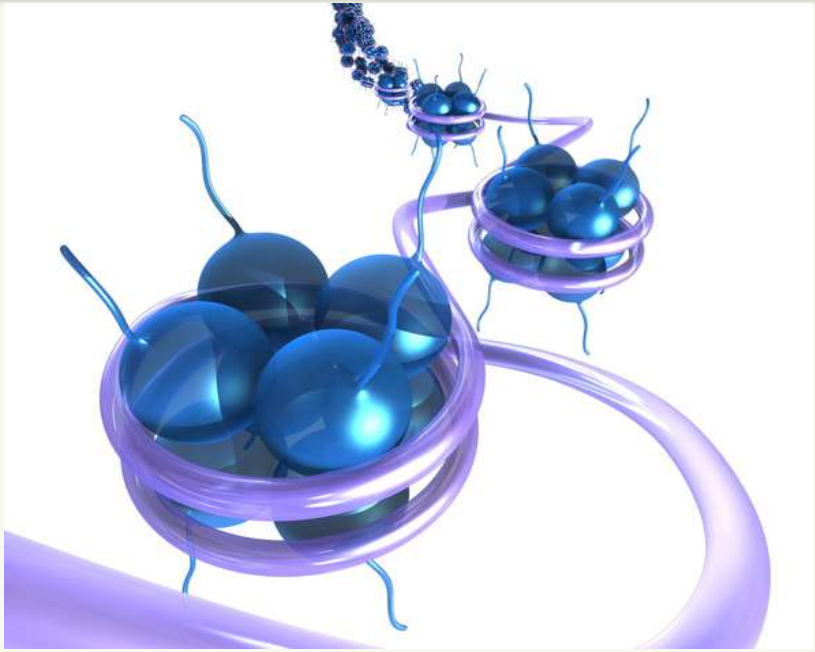


Epigenetics

"Stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence"

from Berger et al. (2009) Genes & Dev. 23: 781-783

© Nicolas Bouvier

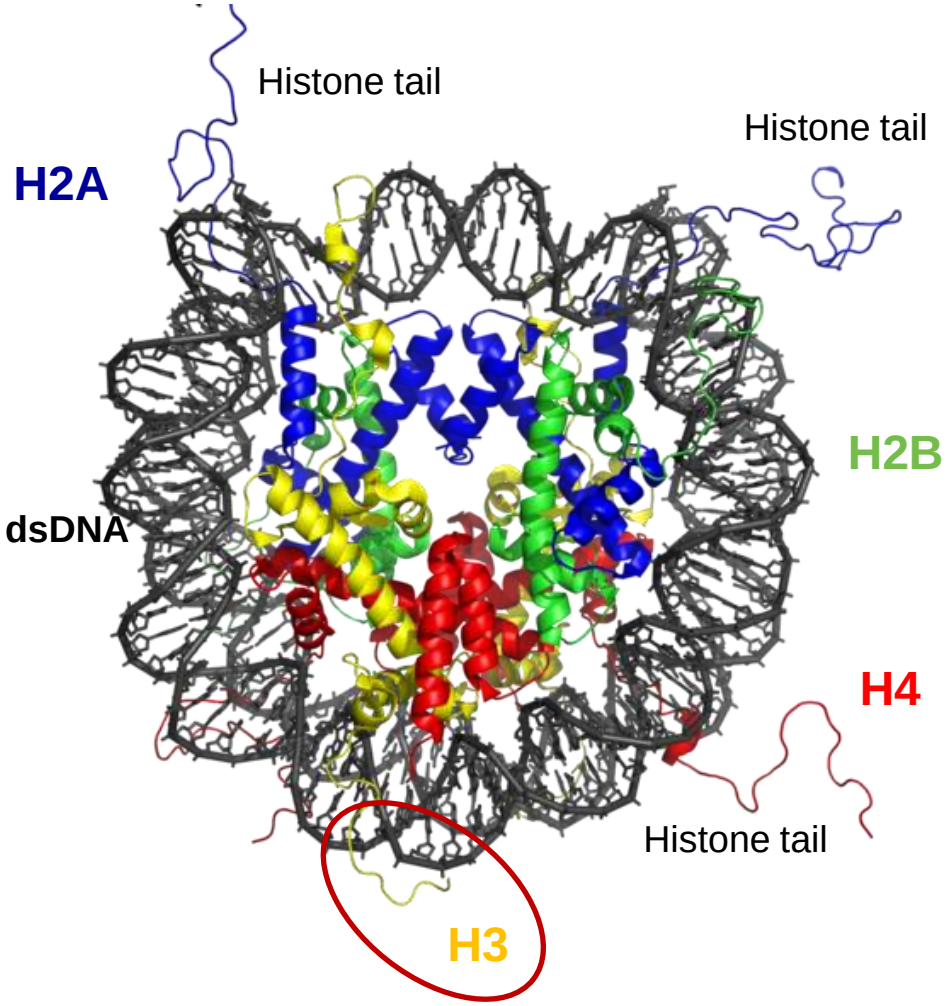
Source: Randy Jirtle, Ph.D., Duke University Medical Center. Used with permission.

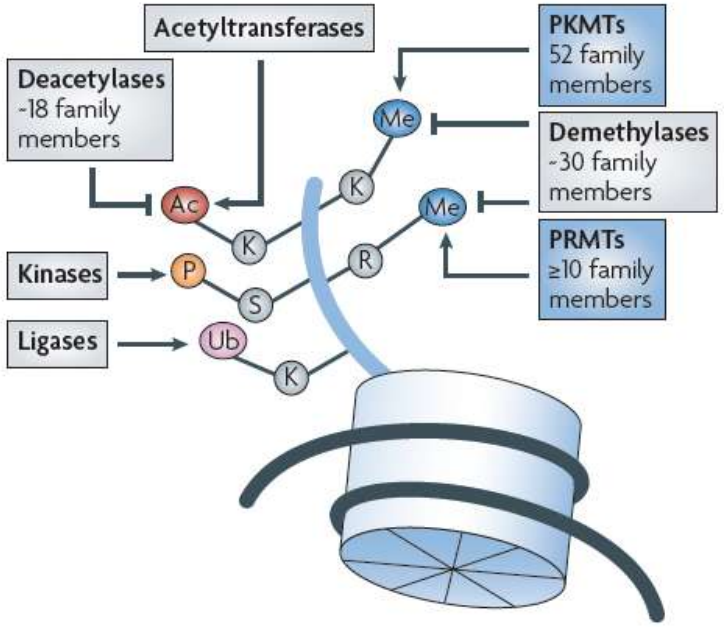
DNA Methylation **& Histone Modification**

Epigenetic Mechanisms – Molecular Base

S/T phosphorylation: mono
K-acetylation: mono
K-methylation: mono, di, tri
R-methylation: mono, di-a, di-s
K-ubiquitination: mono

The Nucleosome:.

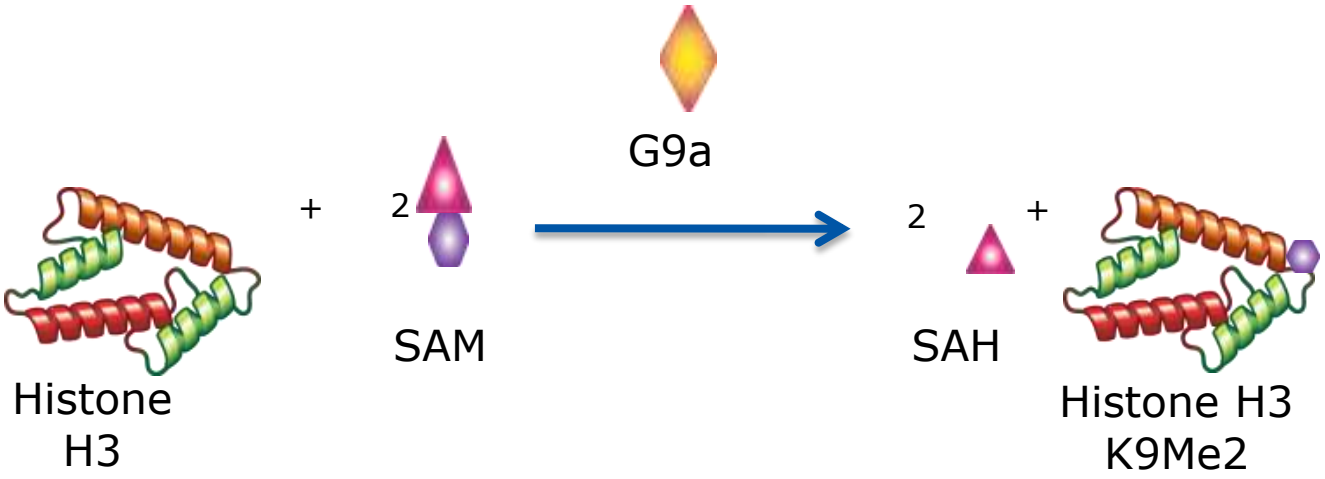




Copeland et al. 2009, Nat.Rev.Drug Disc.

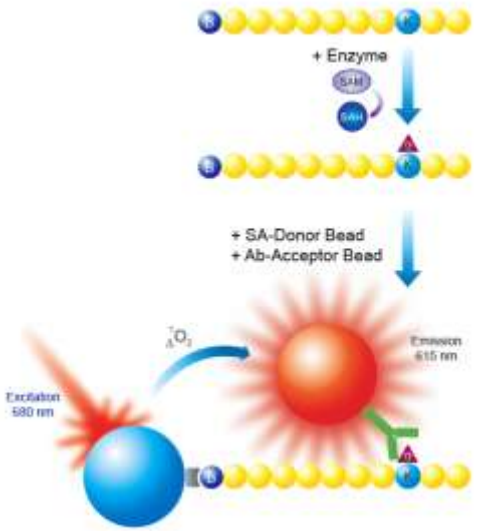
Enzyme assay example: Methyltransferase

Enzymatic Reaction

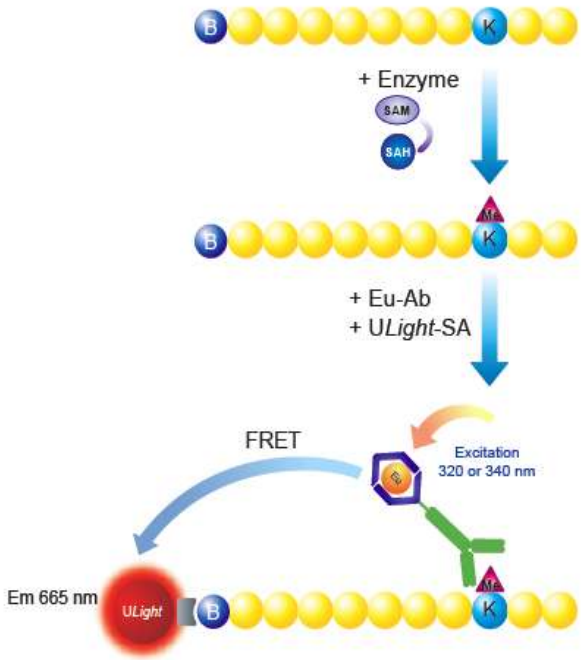


Three ways to set up the assay

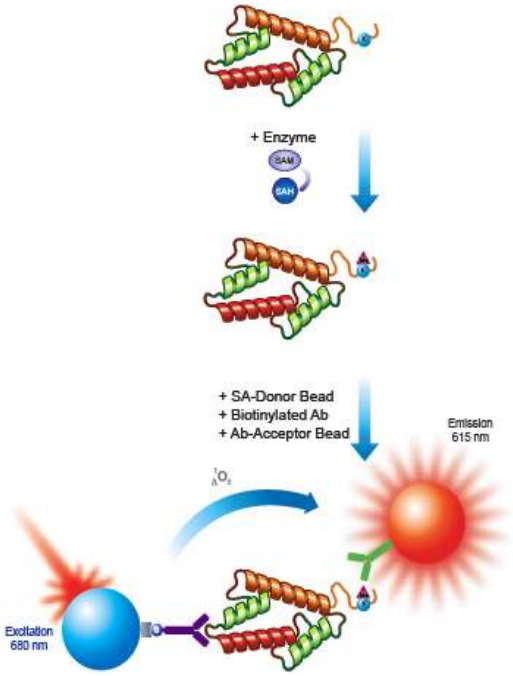
1. Alpha peptide



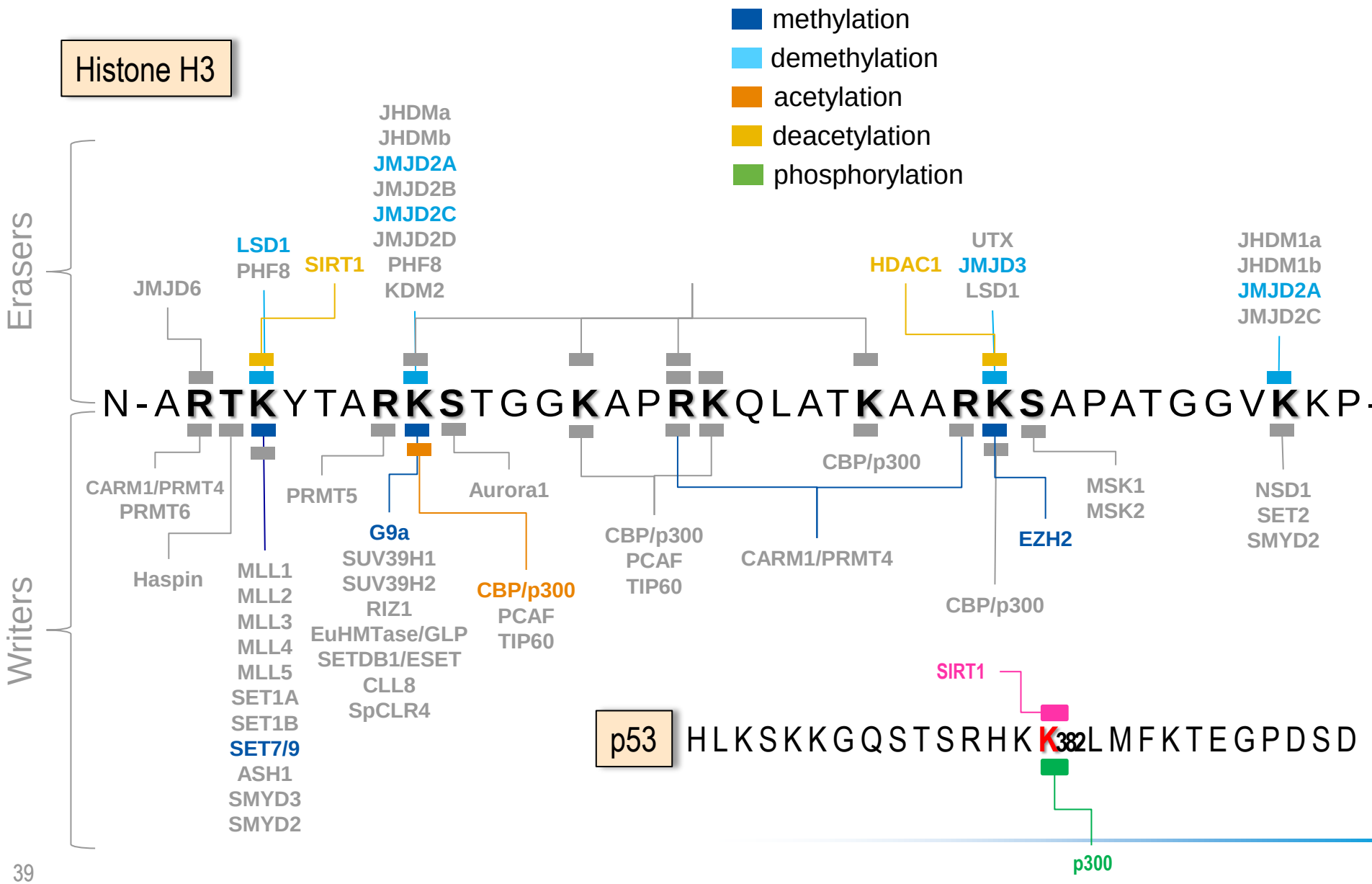
2. LANCE peptide



3. Alpha Full Length

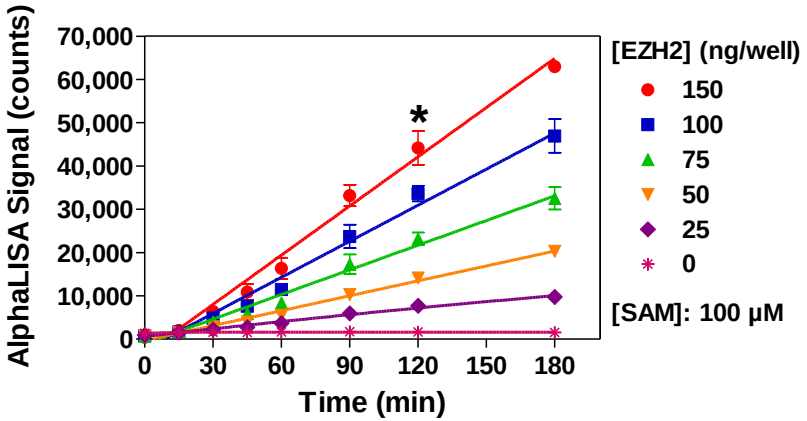


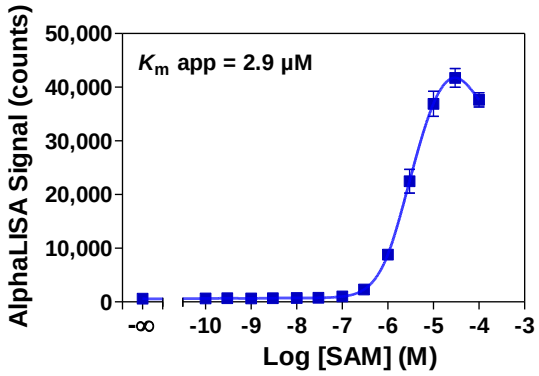
Validated Histone H3 N-terminus Marks & Enzymes

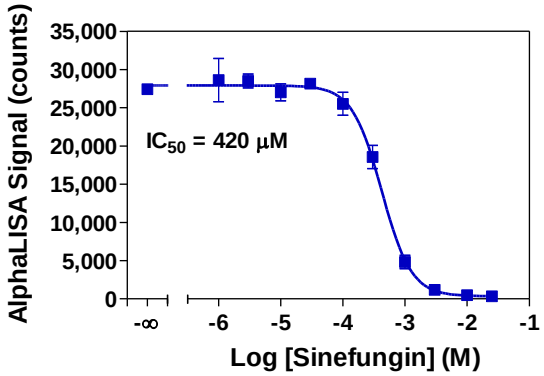


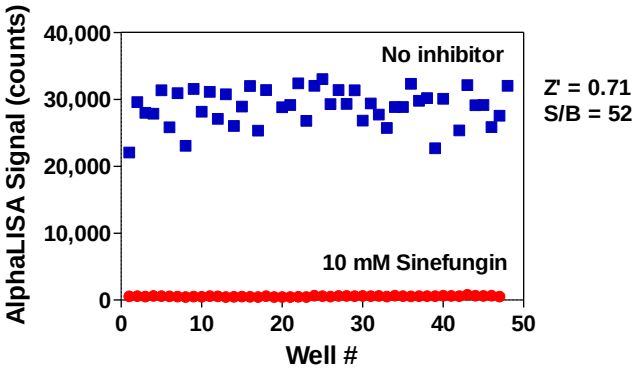
Typical Signal-increase Assay: AlphaLISA EZH2 Methylation Assay

- Optimized assay conditions:
  - 150 ng/well EZH2 complex
  - 100 nM biotin-H3K27me0 peptide (H3 21-44)
  - ATKAAR**K**SAPATGGVKKPHRYRPGGK(Biotin)-OH
  - 3 µM SAM ($K_{m,app}$)
  - 120 min reaction at 23°C (linearity verified)
  - Detection with **anti-H3K27me2-1 Acceptor beads**
  - <1% substrate turnover !!!



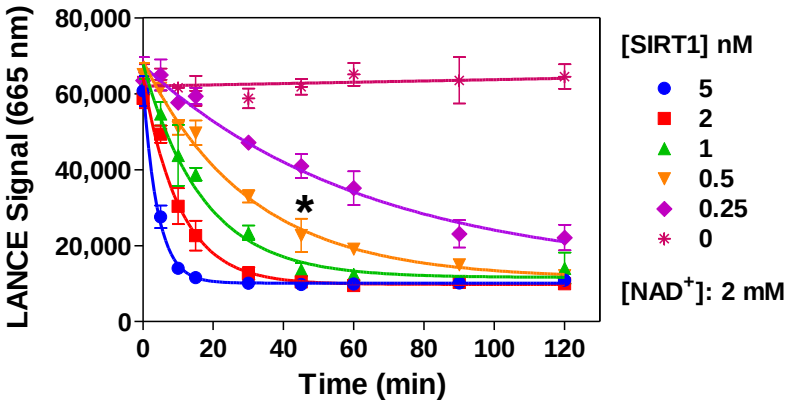


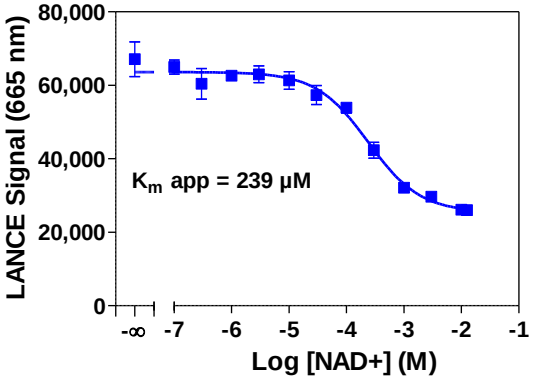


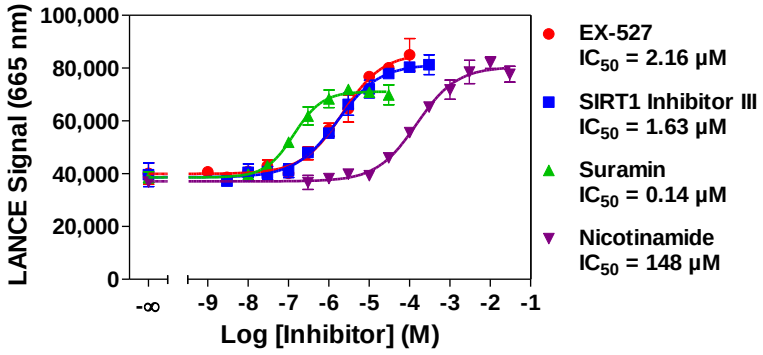


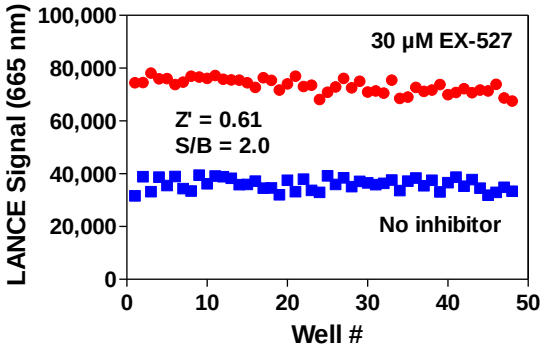
Typical Signal-decrease Assay: LANCE *Ultra* SIRT1 Deacetylation Assay

- Optimized assay conditions:
  - 0.5 nM SIRT1
  - 3 nM biotin-p53K382ac peptide
  - (Biotin)GGHLKSKKGQSTSRHK**K(ac)**LMFKTEGPDSD-NH
  - 200 µM NAD$^+$ ($K_{m,app}$)
  - 60 min reaction at 23°C (linearity verified)
  - Detection with **Eu-anti-p53K382ac Antibody**
  - 50% substrate turnover (S/B of 2)
  - Note: U*Light*–SA can be used at 1 nM









Discovery Solutions for Epigenetic Enzyme Activity

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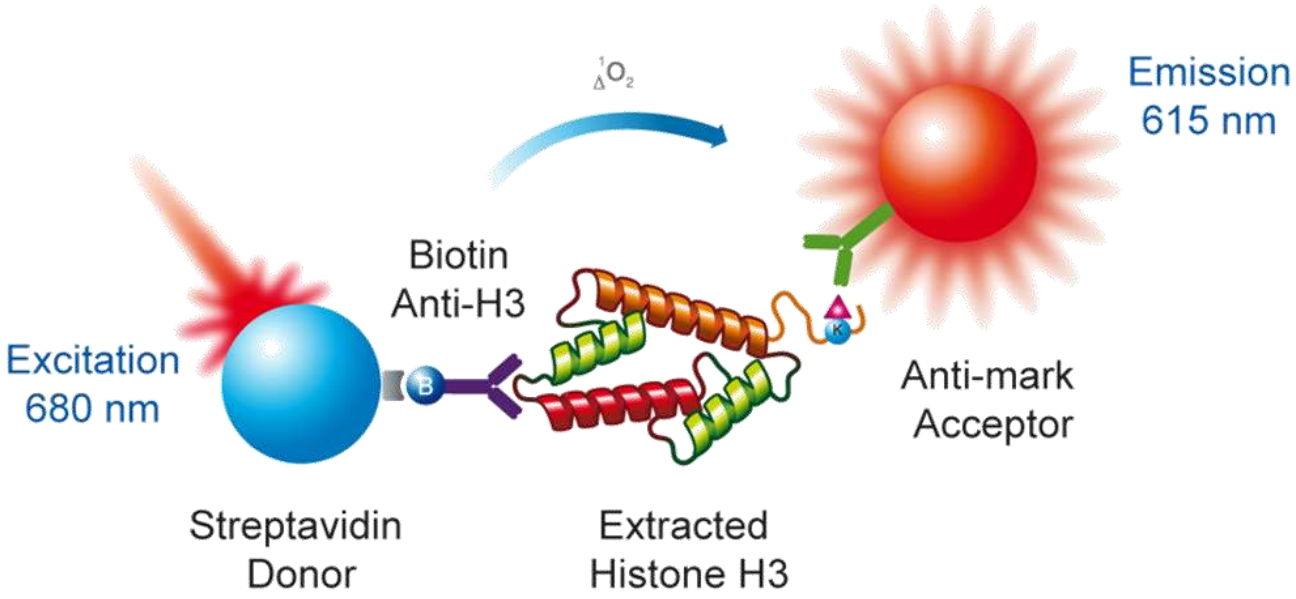
Methyl Modifications		
Mark Detected	**Validated Assays**	
	Methyltransferase (substrate)	Demethylase (substrate)
H3K4 unmodified *NEW!*		**LSD1** (bio-H3K4me1)
H3K4me1-2	**SET7/9** (bio-H3 (1-21) unmodified) **SET7/9** (full length histone H3 substrate)	
H3K9me2	**G9a** (bio-H3 (1-21) unmodified)	**JMJD2A** / **JMJD2C** (bio-H3K9me3 peptide)
H3K27me2-1 *NEW!*	**EZH2** (bio-H3K27me0)	**JMJD3** (bio-H3K27me3)
H3K27me3 *NEW!*	**G9a*** (bio-H3K27me0 or me1)	
H3K36me2 *NEW!*		**JMJD2A** (bio-H3K36me3)
Acetyl Modifications		
Mark Detected	**Validated Assays**	
	Acetyltransferase (substrate)	Deacetylase (substrate)
H3K4 unmodified *NEW!*		**SIRT1** (bio-H3K4ac)
H3K9ac	**p300** (bio-H3 (1-21) unmodified)	
H3K27ac *NEW!*	**P300*** (bio-H3 (1-21) unmodified)	**HDAC1** (signal decrease assay) (bio-H3K27ac)
Non-Histone Target		
p53 K382ac *NEW!*	**p300*** (bio-p53K382 unmodified)	**SIRT1** (signal decrease assay)

Specific & Validated

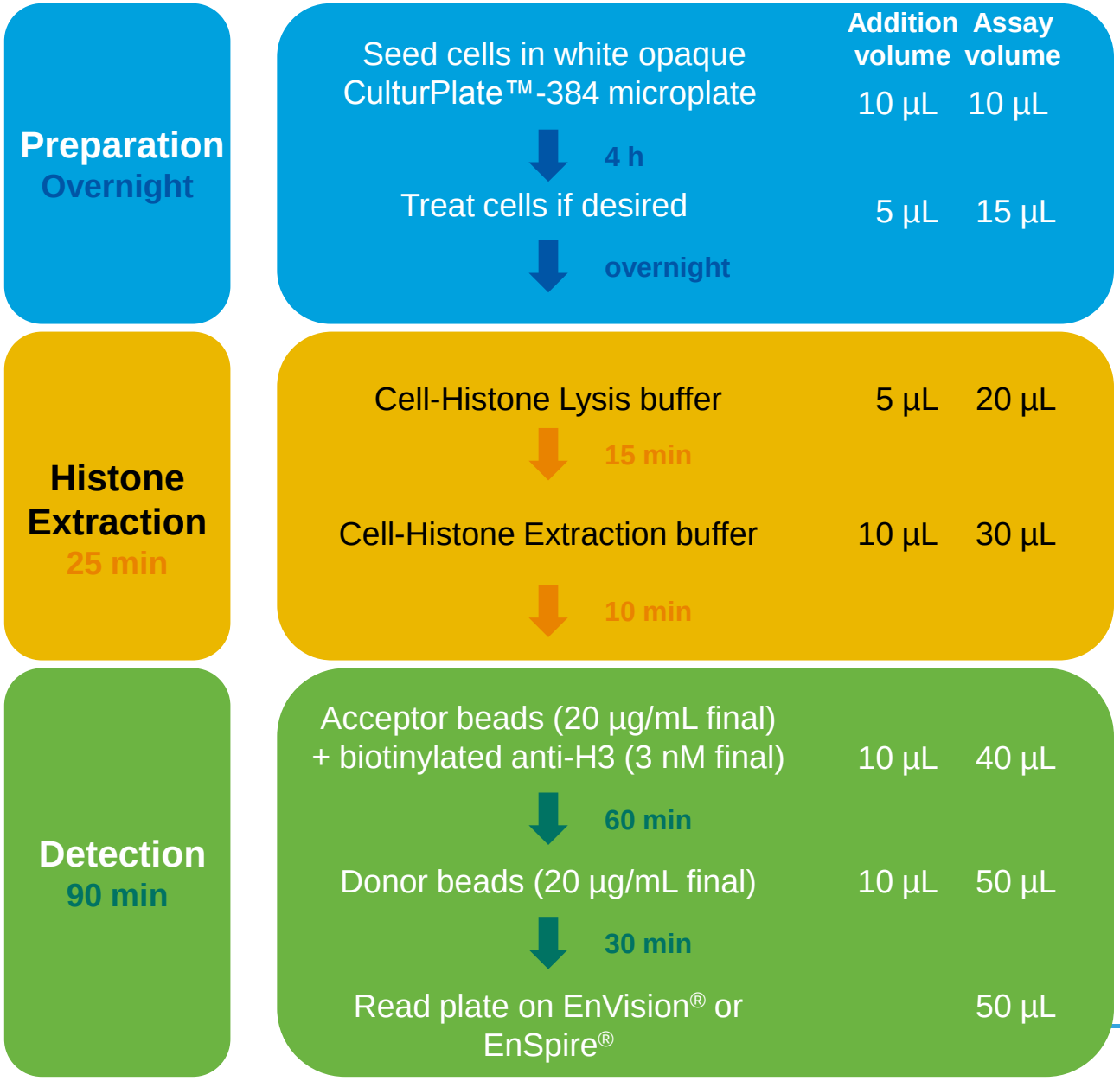
Assay Principle



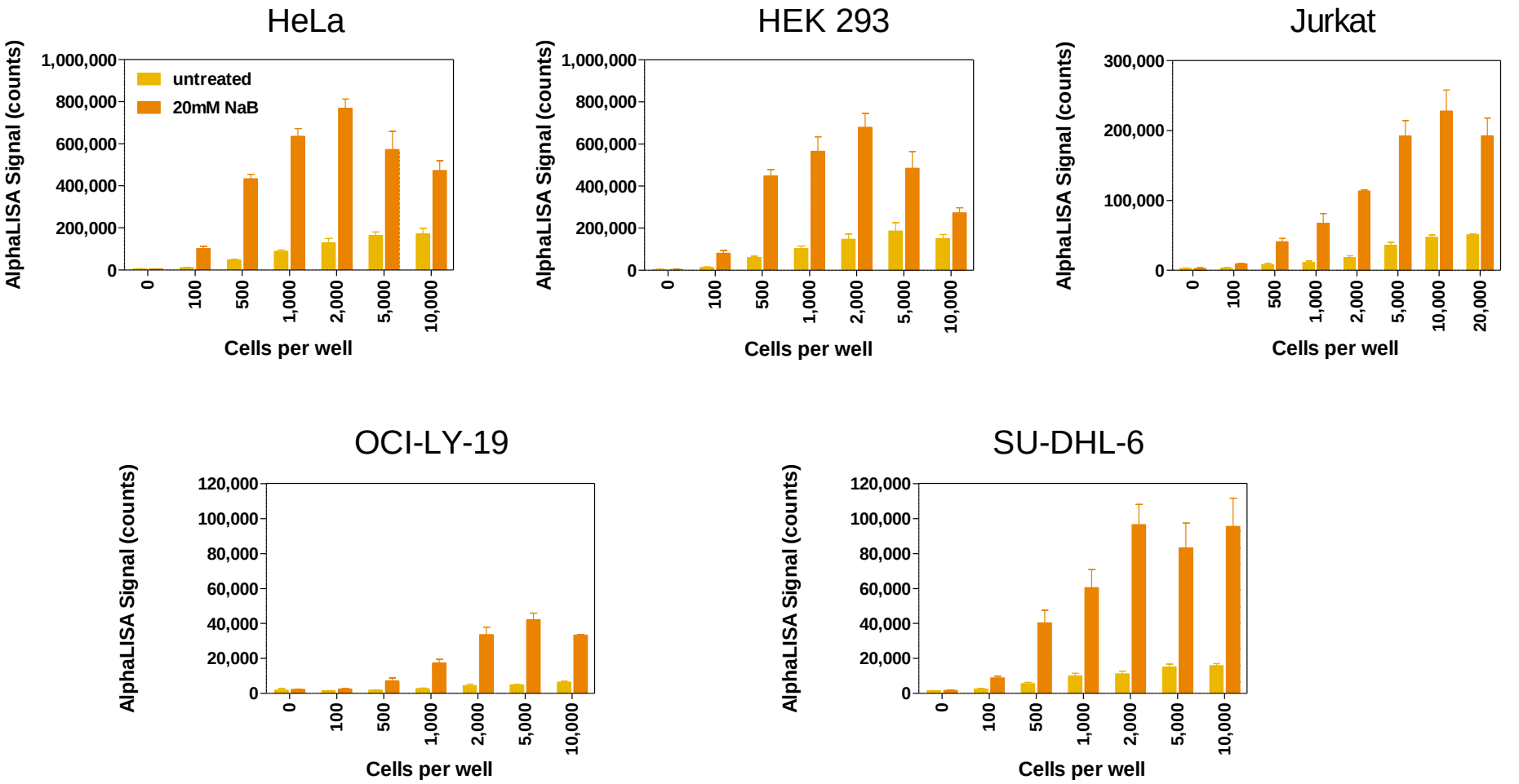
- Cells are grown for at least 18 h w/ and w/o compound
- Cells are lysed with the Cell-Histone Lysis buffer
- Histone are extracted from nucleosomes with the Cell-Histone Extraction buffer
- Histone carrying specific epigenetic marks are captured using the anti-mark Acceptor beads and biotinylated anti-Histone H3 (C-ter) antibody diluted in Cell-Histone detection buffer
- Donor beads are added
- Signal is detected with an Alpha reader

Universal Protocol

- All-in-one-well
  - No transfer steps
- All cell types
  - Adherent
  - Suspension
- No wash steps necessary

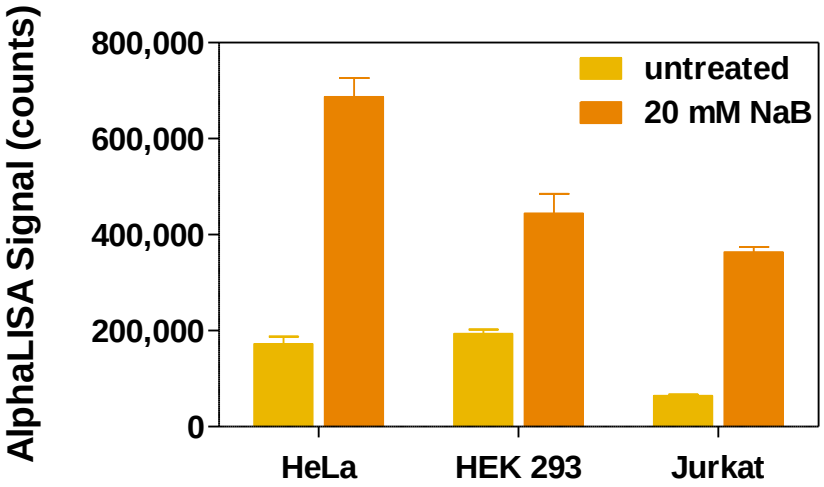


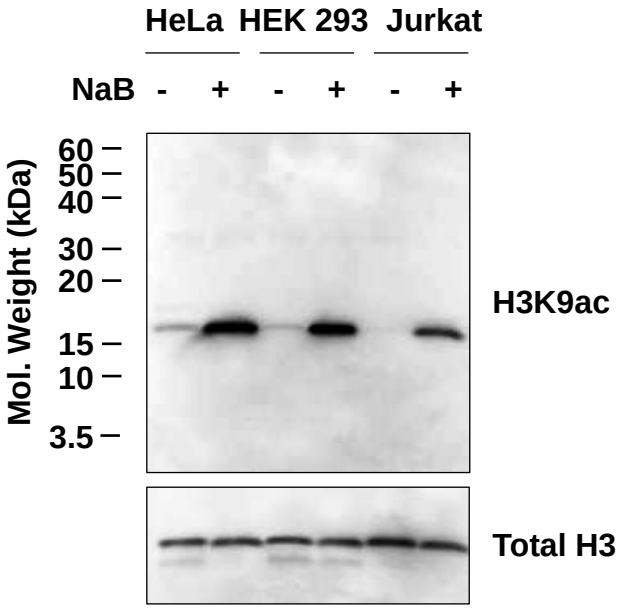
Cell Titration Assay



- Signal increase detected with as few as
  - 500 untreated cells/well
  - 100 NaB-treated cells/well

Selecting the Right Cell Line





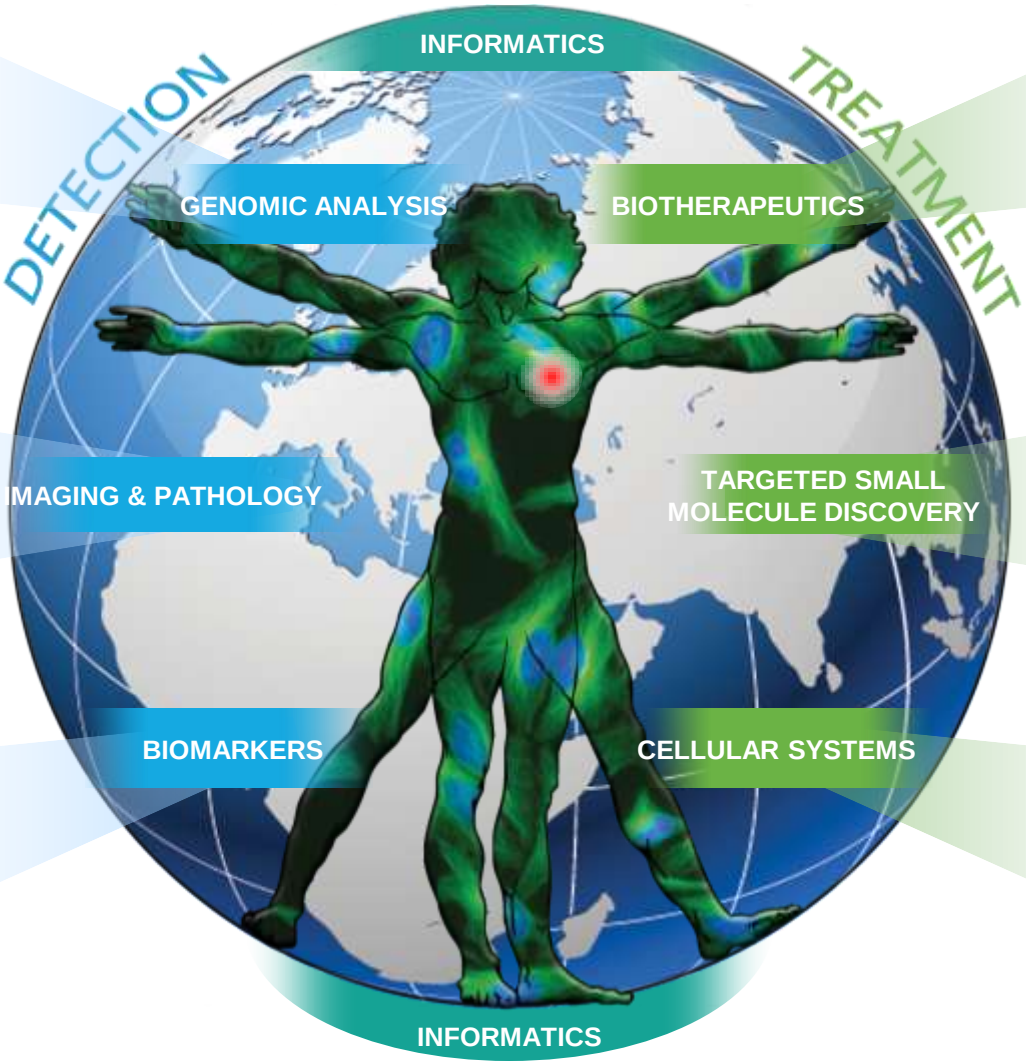
- Different cell lines exhibit
  - different mark levels
  - different NaB-fold stimulation
- Corroboration of Alpha and Western blot data

PerkinElmer Epigenetic Offering

	Histone Mark	Detection	
		Biochemical	Cellular
Acceptor beads Cellular detection kits	H3K4	✔	
	H3K4me1-2	✔	
	H3K4me2		✔
	H3K9ac	✔	✔
	H3K9me2	✔	
	H3K27ac	✔	✔
	H3K27me2	✔	
	H3K27me3	✔	✔
	H3K36me2	✔	
bio-Ab	Histone H3 (C-ter)	✔	✔
Buffers	Epigenetics Buffer 1 Kit (5X)	✔	
	Cell-Histone™ Buffer Set		✔

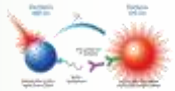
Life Sciences & Technology

LIFE SCIENCES & TECHNOLOGY

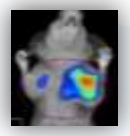




Services, Reagents & Informatics



Discovery & QC Assays

Detection & Probes


Readers & Reagents

Channel to Diagnostics


HCS Imaging & consumables