PerkinElmer
For the Better

HUMAN HEALTH | ENVIRONMENTAL HEALTH

酶标仪检测原理及应用

July-11- 2013

李军辉

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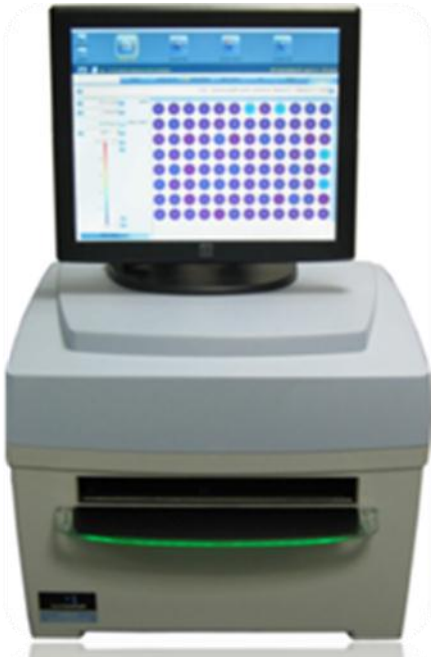
酶标仪的应用

- Agglutination
- Apoptosis
- Bacterial adhesion
- Bacterial identification
- Blue fluorescent protein
- BRET2™
- Cell adhesion
- Cell counting
- Cell expression
- Cell membrane integrity
- and lysis
- Cell proliferation
- Cell viability and cytotoxicity
- Chemotaxis
- Chlorophyll
- Colorimetric assays
- Cytokine analysis
- Cytotoxicity
- DELFIA, preferably
- bRDU assay
- DNA hybridization
- DNA quantification
- (fluorescence and UV
- absorbance)
- ELISA/FELISA
- Endocrine hormones
- Enzyme activity
- Enzymatic cleavage
- Enzyme kinetics
- Gene expression
- Environmental toxins
- FIA
- Gene expression
- Green fluorescent
- protein GFP
- HTS
- Homogeneous TRF
- (LANCE™)
- Immunoassays
- Intracellular Ca2+
- measurement, Fura-2,
- dual-label ratiometric
- and kinetic
- Kinase activity
- LANCE
- Macrophage activity
- Metabolic activity
- MIC (minimum inhibitory
- concentration)
- Mitochondrial membrane
- potential
- Multiprobe assays
- NADH, NADPH
- Na+/H+ exchange
- NK cell activity
- Nucleic acid quantification
- Oxidative burst
- Oxidation reactions
- PCR product (quantitative
- and qualitative)
- pH indicators
- Phagocytosis
- Phospholipid assay
- PKU
- Protease activity
- Protein assays quantification
- Receptor binding studies
- Reporter gene
- Reverse transcriptase
- RNA hybridization
- RNA quantification
- Signal transduction
- SNP (Single Nucleotide
- Polymorphisms)
- Steroid hormones
- Toxicology
- Uranium

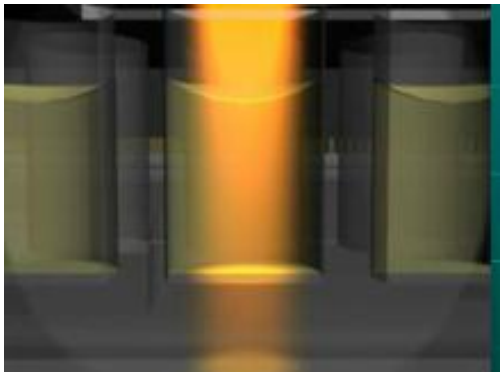
技术原理



- 光吸收
- 化学发光
- 荧光强度
- 荧光偏振
- 时间分辨荧光
- Alpha技术
- Label-free技术

光吸收

- 物质对光的吸收
- 比耳定律 **A=KCL**



K：吸收系数；C：物质的浓度；L：溶液光径长度

- 物质浓度测定

DNA： 260 nm

Protein： 280 nm

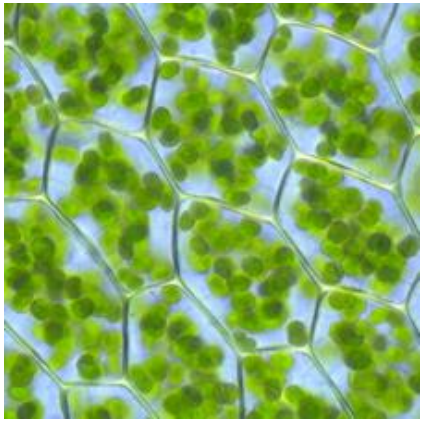
BCA： 562 nm

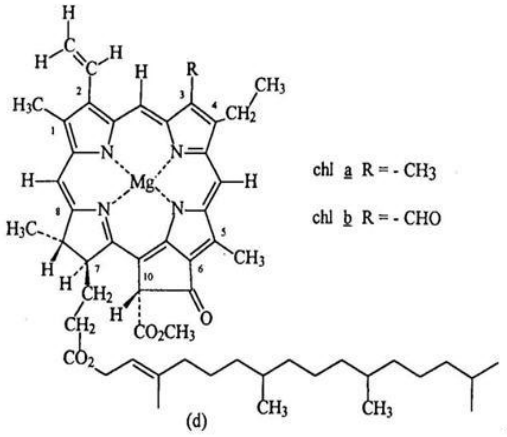
Elisa： 450 nm

MTT： 490 nm

NADH： 340 nm

Bacteria: 600 nm





Chlorophyll: K=10^5 $cm^{-1}M^{-1}$

BSA: 6.67 $cm^{-1}M^{-1}$

IgG: 14 $cm^{-1}M^{-1}$

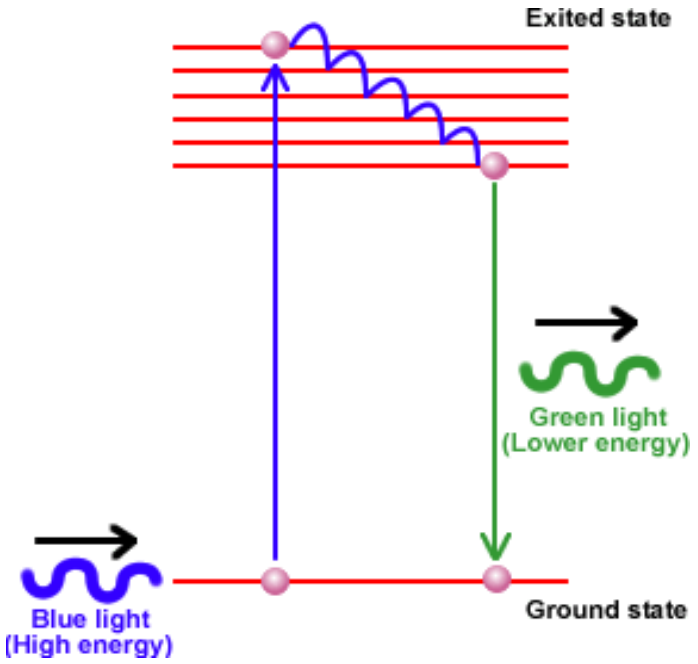
- **Plate： Clear**

荧光

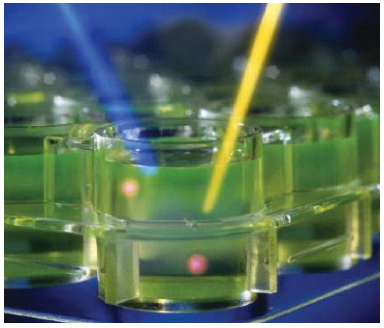
- 能量由光辐射提供激发: 物理激发
- E=*hv*=*hc*/λ

  h：普朗克常量 *v*：频率 c：光速 λ：波长
- E发射<E激发： *hc*/λ发射< *hc*/λ激发

  λ发射>λ激发

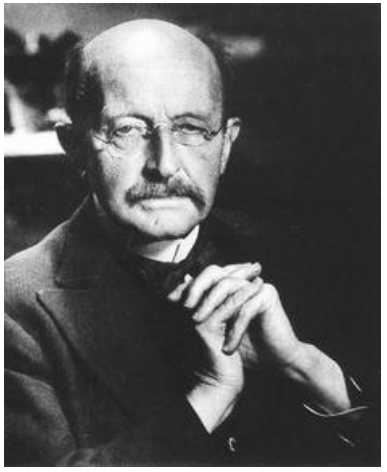


荧光素λ发射=535 nm＞荧光素λ激发=485 nm



Guess?

Niels Bohr



Max Plank

- Plate: Black

荧光应用

- **Ca^{2+} 测定：** Fura-2， Fluo-3
- **DNA定量：** Pico Green
- **RNA定量：** Ribo Green
- **GUS报告基因：**

- **细胞增殖：** Alamar Blue
- **细胞凋亡：**

Ac-DEVD↓AMC
Caspase-3

化学发光

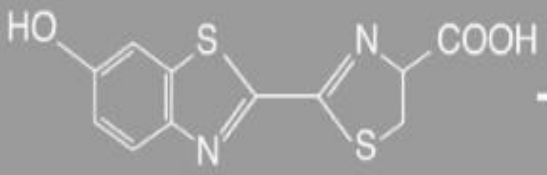
- 能量由化学反应提供激发 ： 化学 激发　A+B=C*

→ C+light

- 常消耗高键能物质如ATP
- 闪光性（flash）、辉光型（glow）
- Plate： White

化学发光应用: Chemistry

Chemistry of Bioluminescence



luciferin

+ O_2 + ATP

luciferase

Mg^{++}

Glo

. . . to create a suite of robust and sensitive biological assays.

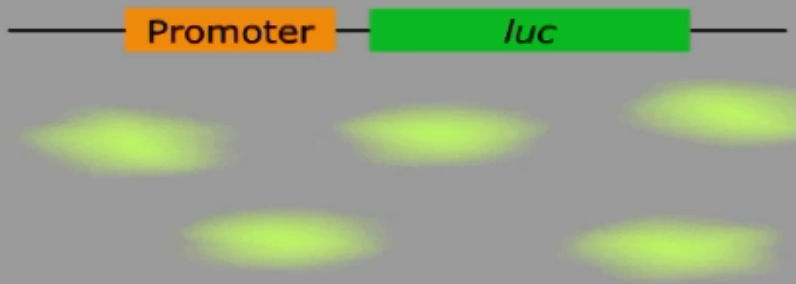
- Genetic Reporter Assays
  Measure changes in luciferase production
- ATP Assays
  Measure changes in ATP levels
- Luciferin Substrate Assays
  Measure enzymatic release of free luciferin

化学发光应用：Luciferase

Genetic Reporter Assays

measure production of luciferase

Strong Promoter



Greater Expression

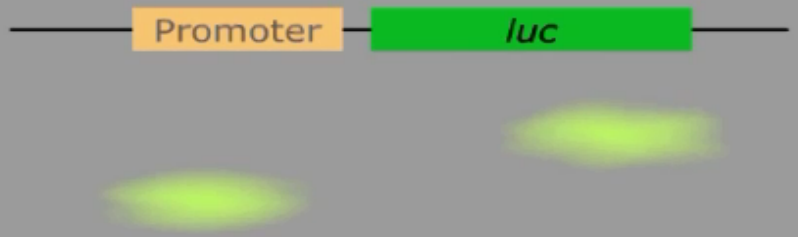
▼

More Luciferase

▼

Greater Luminescence

Weak Promoter



Less Expression

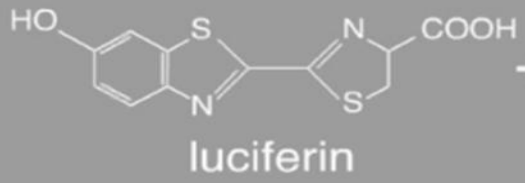
▼

Less Luciferase

▼

Less Luminescence

化学发光应用： ATP-Cell viability



+ O_2 + ATP

luciferase

Mg^{++}

Glo

ATP Assays (cell viability example)
measure changes in ATP levels

Untreated Cells

Treated Cells (Toxic Agent)

More Viable Cells

▼

More ATP

▼

Greater Luminescence

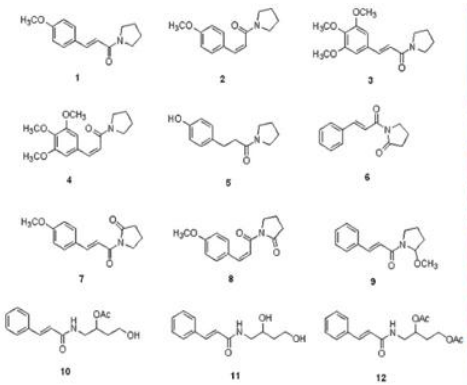
Fewer Viable Cells

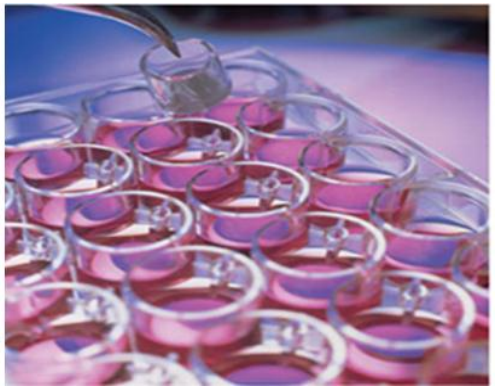
▼

Less ATP

▼

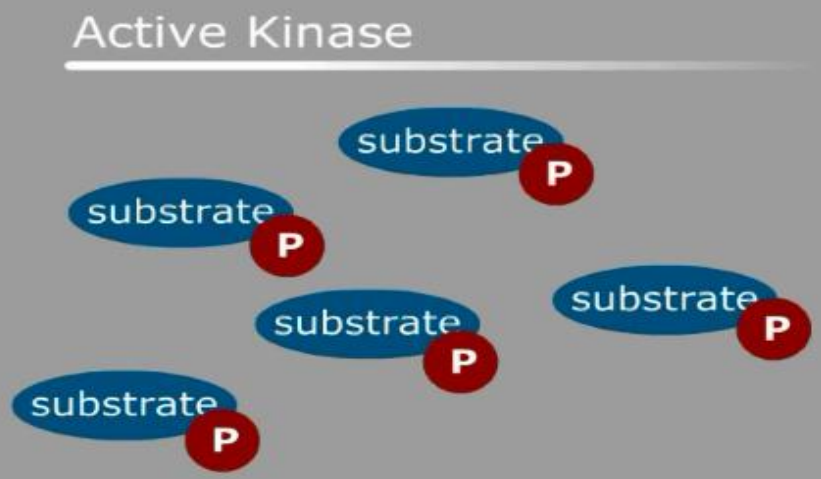
Less Luminescence



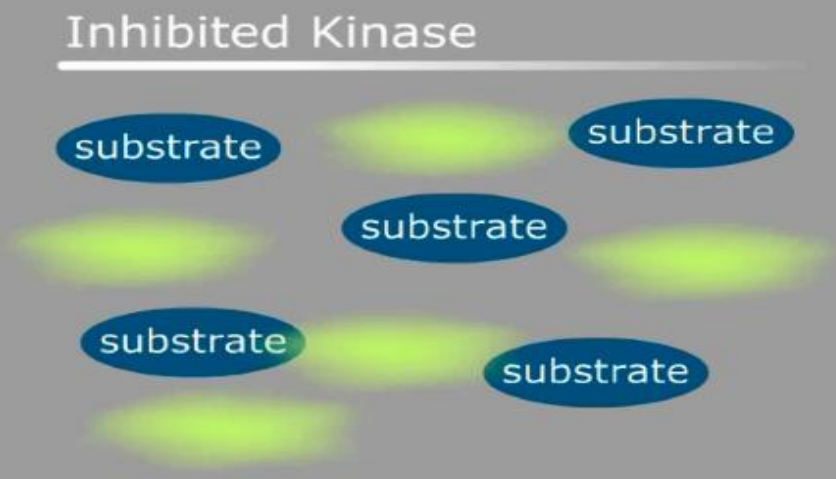


化学发光应用： ATP-Kinase assay

ATP Assays (kinase assay example)
measure changes in ATP levels



Active Kinase
▼
Less Free ATP
▼
Less Luminescence

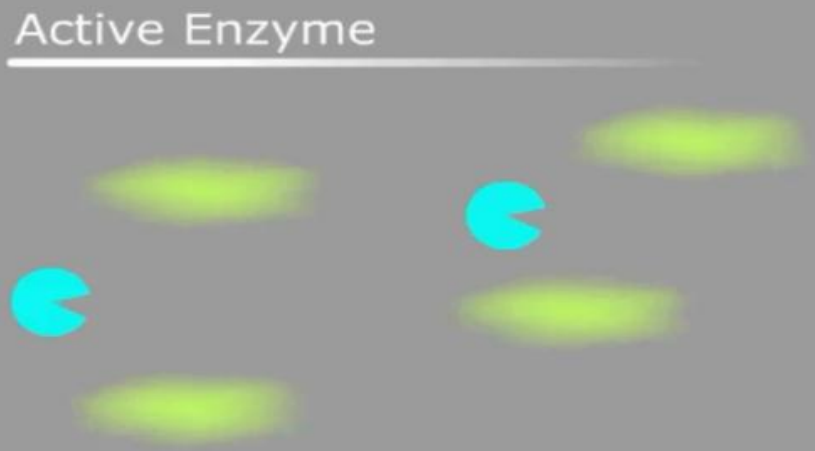


Inhibited Kinase
▼
More Free ATP
▼
Greater Luminescence

化学发光应用：Luciferin-P450 (ADME)

Luciferin Substrate Assays

measure enzymatic release of free luciferin



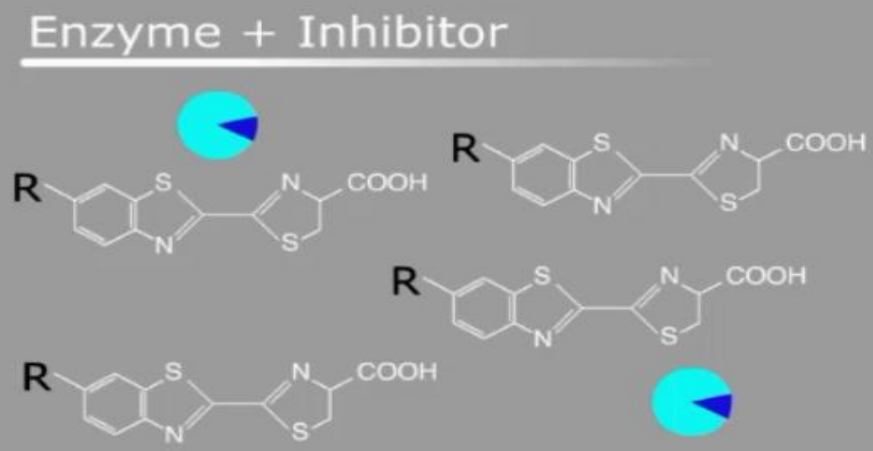
Active Enzyme

▼

Generates Free Luciferin

▼

Greater Luminescence



Inhibited Enzyme

▼

Less Free Luciferin

▼

Less Luminescence

时间分辨荧光：“剩”者为王

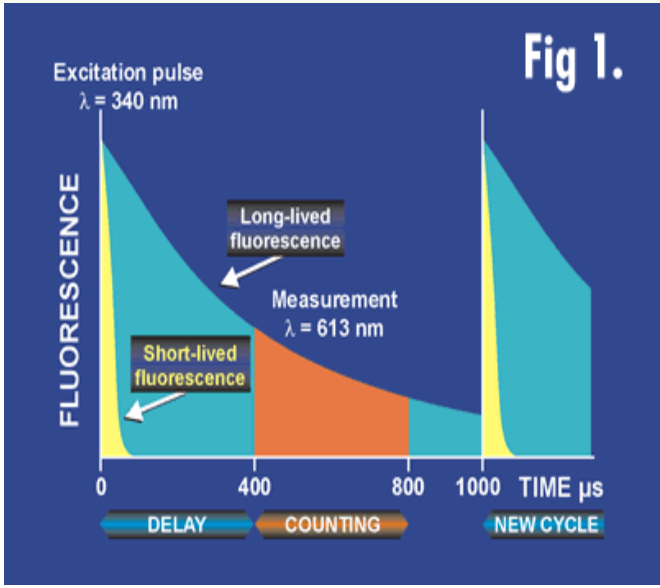
❖ 镧系元素：Eu、Sm、Tb、Dy

Double L

❖ **Long half-life：** 可达ms

❖ **Large Stoke’s shift**

Eu	320	615
Sm	320	642
Tb	320	545
Dy	320	572

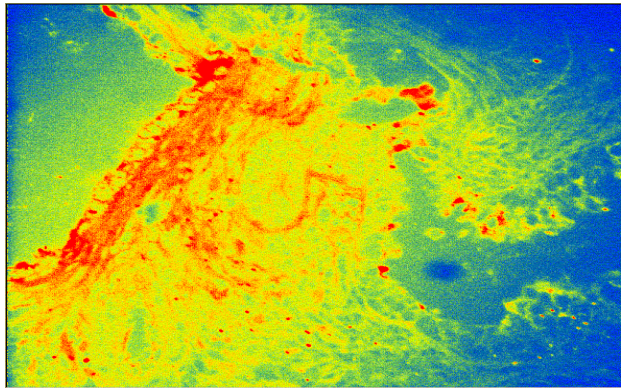


Double D

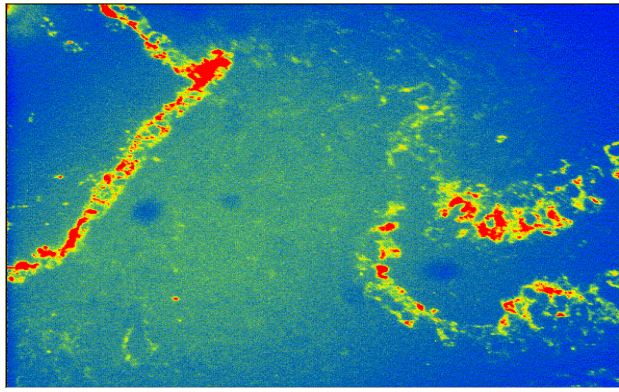
❖ **Delay**

❖ **Decay**

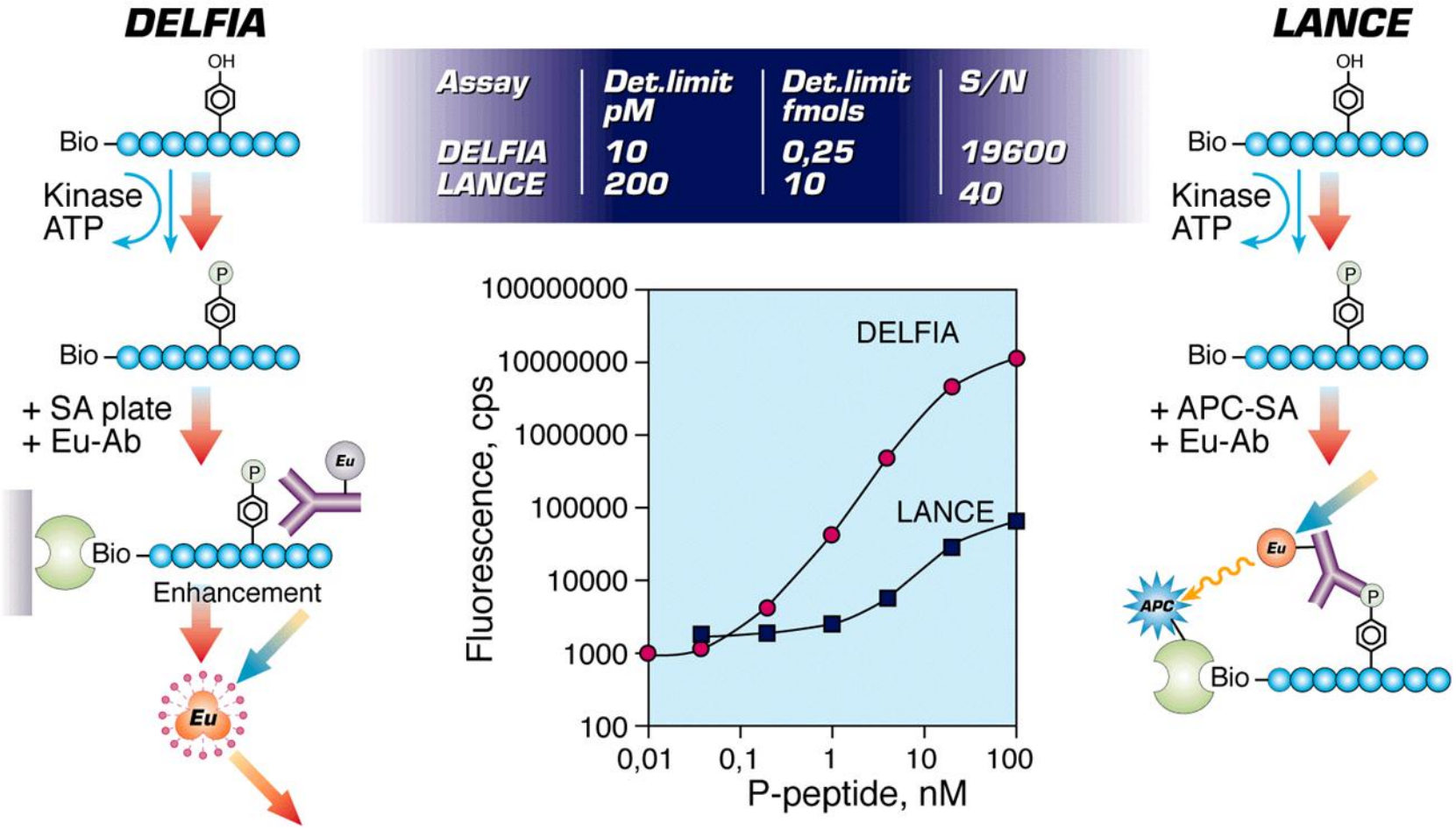
Prompt signal
立即检测的信号



Time resolved signal
时间延迟后的信号



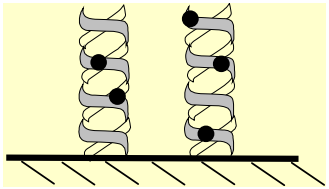
时间分辨荧光应用


DELFIA
OH
Bio
Kinase
ATP
P
Bio
+ SA plate
+ Eu-Ab
Eu
P
Bio
Enhancement
Eu
Assay | Det.limit pM | Det.limit fmols | S/N
DELFIA | 10 | 0,25 | 19600
LANCE | 200 | 10 | 40
100000000
10000000
1000000
100000
10000
1000
100
Fluorescence, cps
DELFIA
LANCE
0,01
0,1
1
10
100
P-peptide, nM
LANCE
OH
Bio
Kinase
ATP
P
Bio
+ APC-SA
+ Eu-Ab
Eu
APC
P
Bio

DELFIA Proliferation protocol

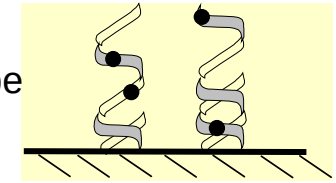
Cell culture
(1 – 5 days,
+37°C)

BrdU Labeling
(2 – 24 h, +37°C)

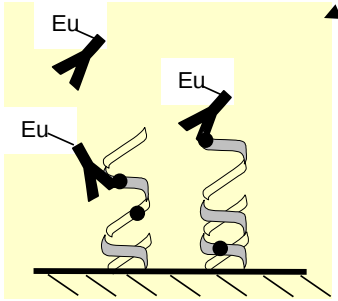


Remove labeling medium
(suspension cells have to be
centrifuged)

Cell fixation and
denaturation
(30 min, RT)

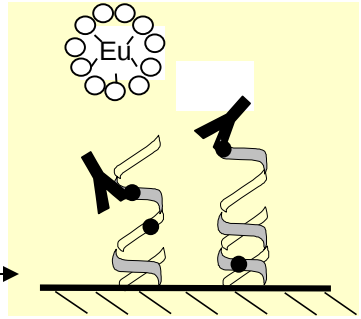


Remove Fix Solution



Wash 4 times

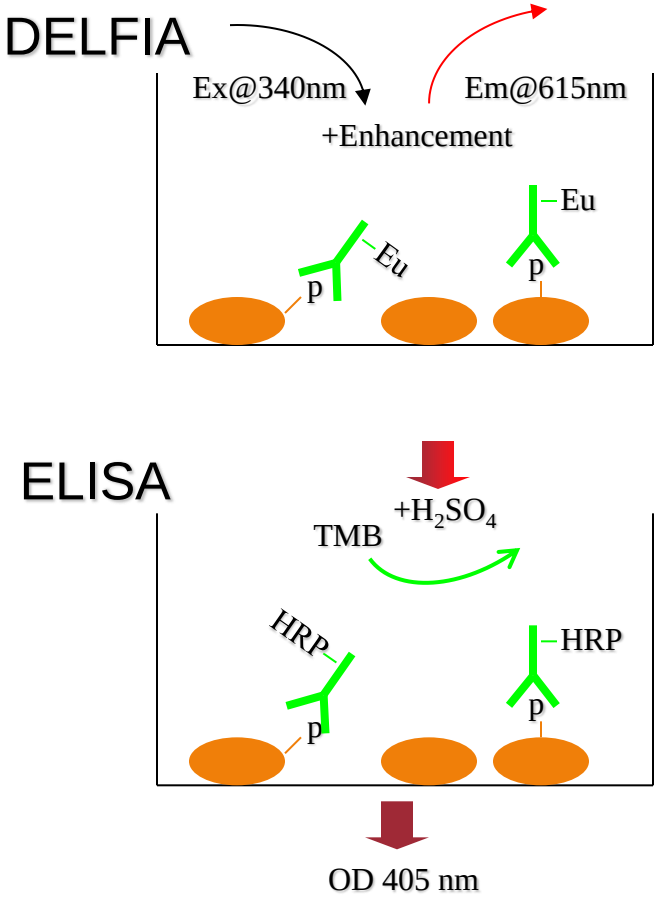
Add DELFIA Inducer
and incubate 15 min

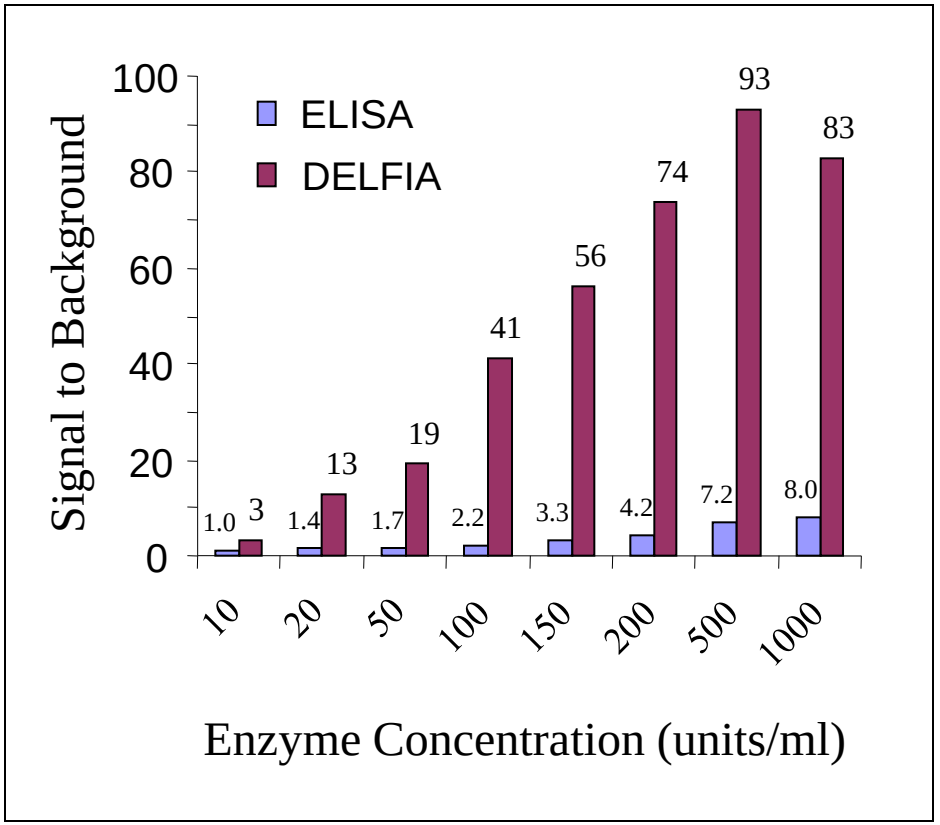


Eu-Anti-BrdU
incubation
(30 – 120 min, RT)

Measure TR-fluorescence

An attractive alternative to Elisa





www.iscpubs.com/pubs/abl/articles/b9812/b9812log.pdf

Another useful reference for DELFIA kinase assay:
Gaarde WA, etc., "Development of a Nonradioactive, Time-Resolved Fluorescence Assay for the Measurement of Jun N-terminal Kinase Activity *J Biomol Screen* 1997 Aug 2:4 213-23

时间分辨荧光应用

TruPoint

Helicase

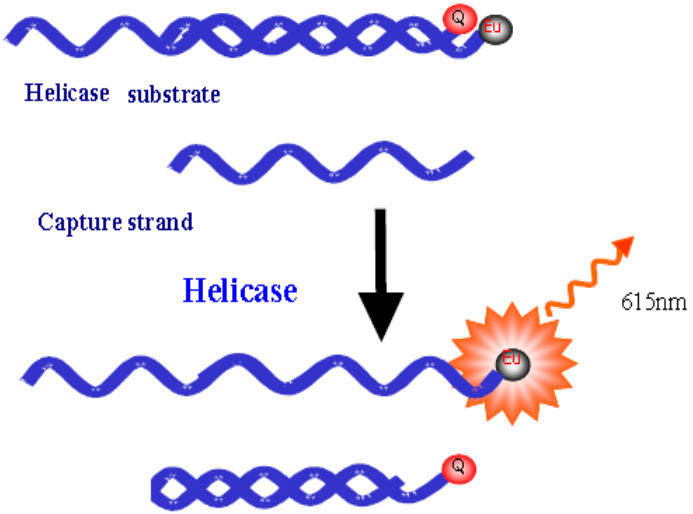
- Cell cycle
- Tumor

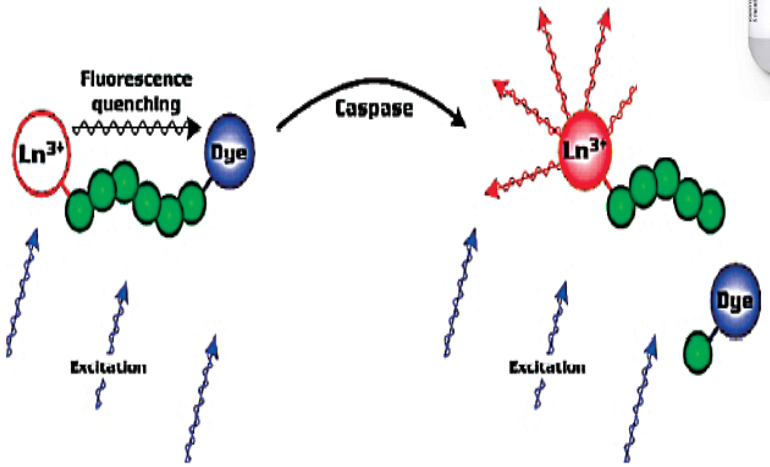
Protease

- HIV-protease
- HCV-protease
- Caspase
- Osteoporosis

Telaprevir

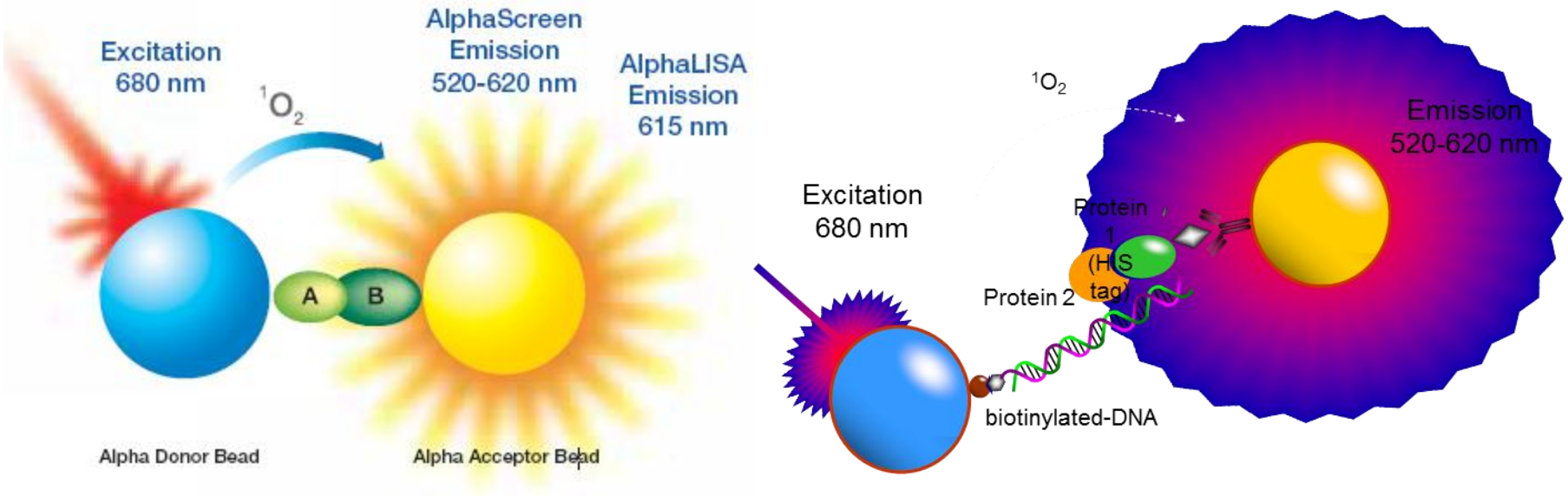
Boceprevir





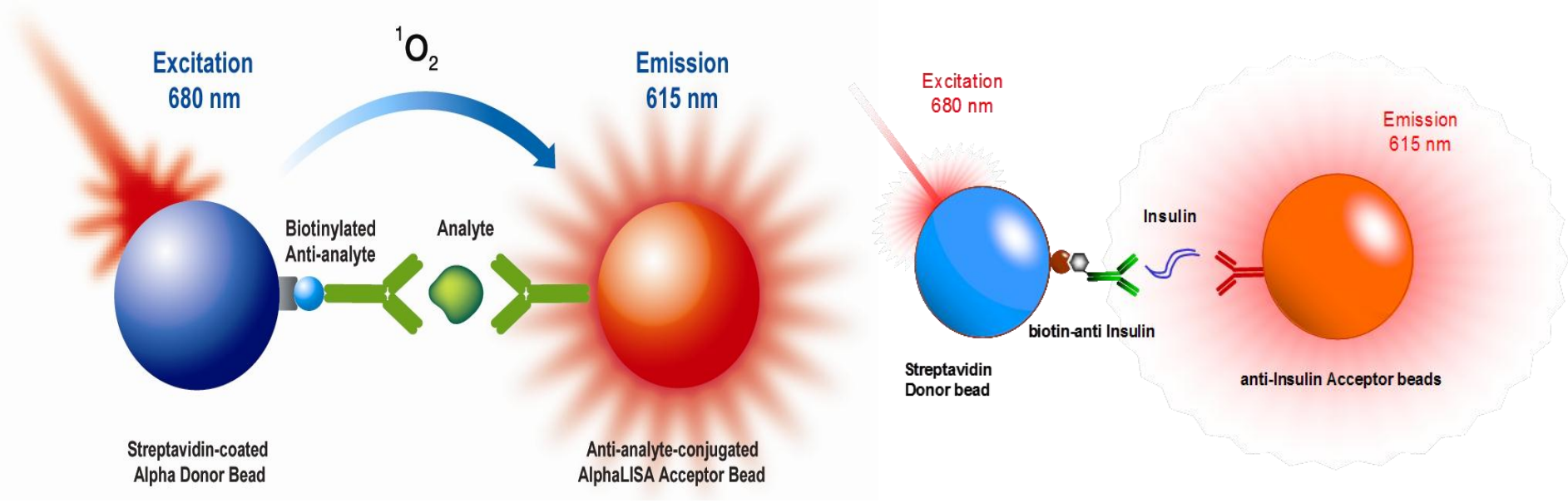
Alpha技术: AlphaScreen

分子间相互作用

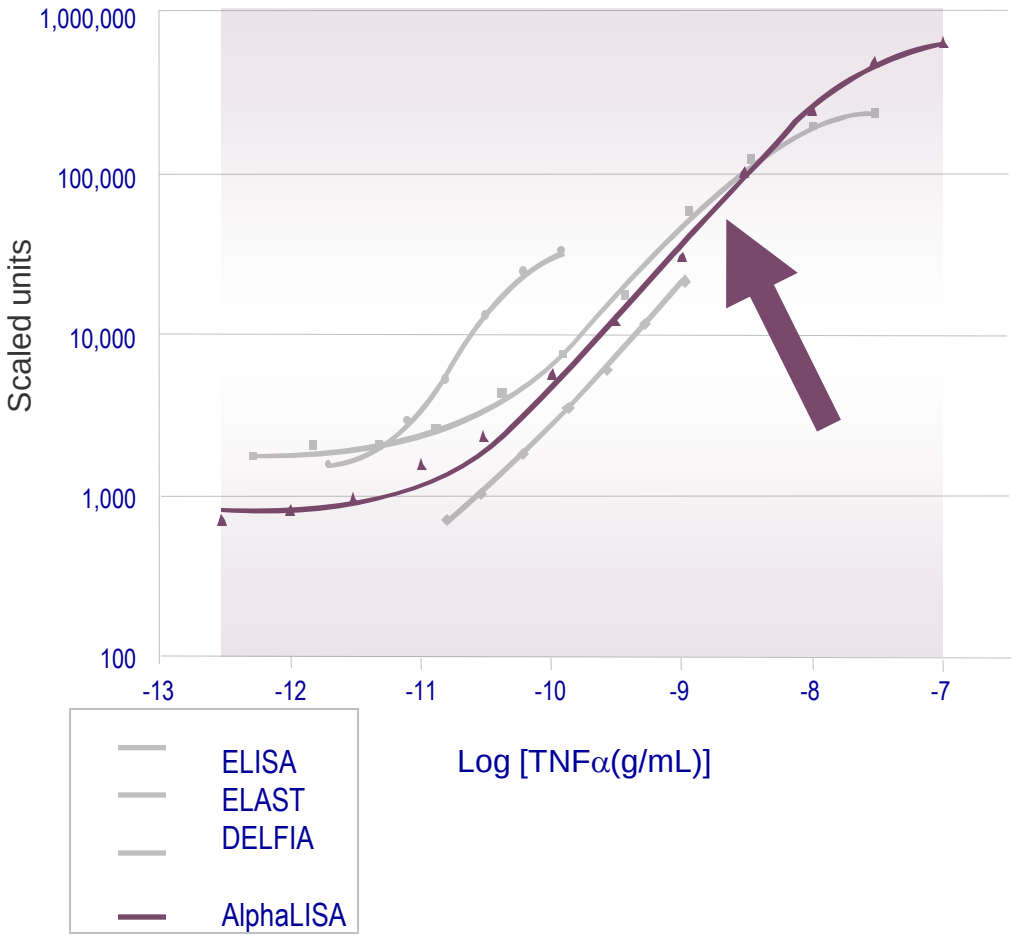


AlphaLISA

复杂样品中痕量物质定量



AlphaLISA优点



灵敏度高

- 节省资金
- 降低对样品用量的要求

动态范围宽

- 无需稀释

均相性技术

- 缩短工作时间
- 缩短发表时间
- 减少样品制备时间

亲近性检测

- 简单到复杂的生物学相互作用
- 血清和血浆来源

AlphaLISA vs ELISA

	传统的 ELISA	AlphaLISA
特异性	高	高
灵敏度	10^{-10} 摩尔 / 孔	10^{-17} 摩尔 / 孔
检测性质	非均相：需要至少 6 个步骤洗涤	均相：无需洗涤
高通量	96 孔板	96/384/1536/3456 孔板均可
抗体要求	需要高亲和力抗体	高 / 低亲和力抗体均可应用
样品体积	大：25-50μL	小：5μL
检测范围	2 个数量级	3-5 个数量级
所需仪器	标准微孔板读数议	EnVision Alpha 或 Enspire Alpha

AlphaLISA® Immunoassay Kits

Biologics	Angiogenesis	Cancer	Cardio-vascular	Inflammation	Metabolic	Neuro-degeneration
IgG	**TNF alpha**	EGF-R	EPO	COMP	Adiponectin	**Aß 1-40**
CHO-P	VEGF	EPO	Myeloperoxidase	G-CSF	GH	**Aß 1-42**
NSO-P	EPO	PSA	NT-proBNP	GM-CSF	GLP-1	sAPPα
	VEGFB	**TNF alpha**	Plasminogen	IFN gamma	Insulin	sAPPβ
	VEGFC	AFP	Renin	IL10	Leptin	
	VEGFD	EPO-R	tPA	IL17	Prolactin	
		ERBB2 / HER2		IL1ß	IGF1	
		MMP1		IL2	IGF2	
		MMP9		IL3		
		b-NGF		IL6		
				IL8		
				TNF alpha		
				CRP		
				IL1α		
				IL12 (p70)		
				IL13		
				IL18		
				CCL2 / MCP1		

Virology
HIV p24

Red = NEW kits

AlphaLISA-Life is totally different!

总结

光吸收	物质对应单波长
化学发光	样品自身发光
荧光强度	激发与发射双波长
荧光偏振	结合值高，离散值低
时间分辨荧光	延迟检测，降低背景
Alpha技术	替代Elisa
Label-free	折射率反应结合