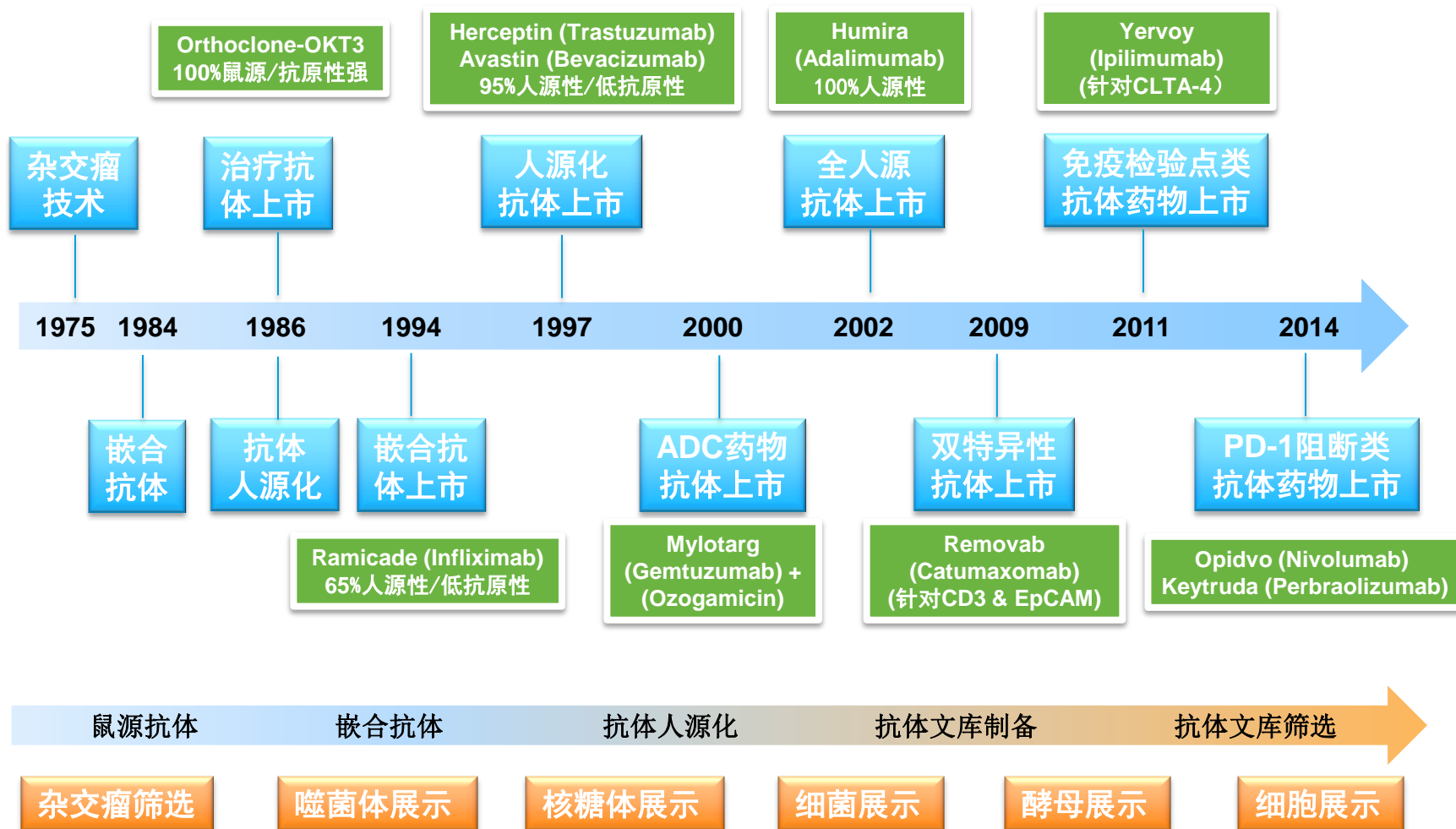




PerkinElmer Biologics Solution

Amy Zhang 张薇 amy.zhang@perkinelmer.com

抗体药物及抗体制备技术发展

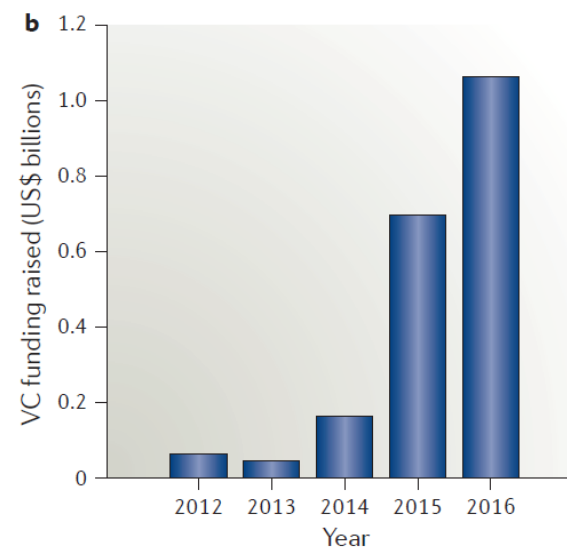
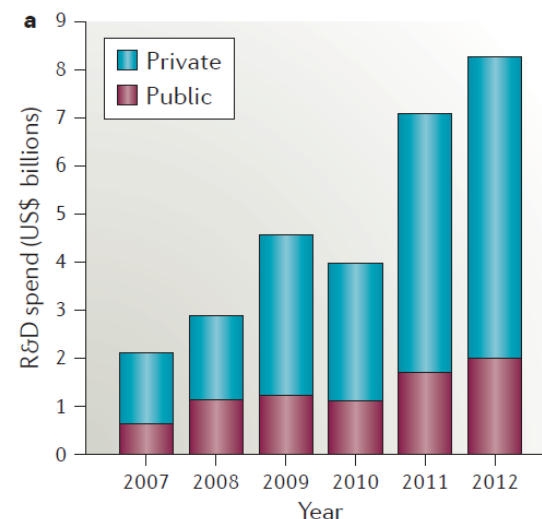


Chinese biopharma starts feeding the global pipeline

Table 1 | Select list of drugs that were discovered in China

Drug name	Sponsor	Properties	Lead indication	Status
Chidamide	Chipscreen	HDAC inhibitor	T cell lymphoma	CFDA approved in 2015, global phase I/II trials ongoing
Fruquintinib	Chi-Med/Lilly	VEGFR inhibitor	Colorectal cancer	NDA filed with CFDA, and global phase I expected soon
Savolitinib	Chi-Med/AstraZeneca	MET inhibitor	Papillary renal cell carcinoma	Global phase III to start this year
BGB-3111	BeiGene	BTK inhibitor	Waldenström macroglobulinaemia	Global phase III and China pivotal trials ongoing
BGB-A317	BeiGene	Anti-PD1 mAb	Cancer	Global phase I and China pivotal trials ongoing
BGB-283	BeiGene	RAF dimer inhibitor	Cancer	Global phase I ongoing
Emibetuzumab	Innovent Biologics/Lilly	Anti-MET mAb	NSCLC	Global phase II ongoing
MAK683	Novartis	EED inhibitor	B cell lymphoma	Global phase I/II ongoing
RG7854	Roche	TLR7 agonist	HBV	Global phase I ongoing
RG7097	Roche	Capsid inhibitor	HBV	Global phase I ongoing

Biopharma investment in China.



生物大分子制药研发流程

加快
研究速度

提高
精准治疗

高内涵细胞显微成像系统
HCS Opera Phenix

临床前活体影像系统
IVIS Spectrum

高通量多模式检测系统
EnVision HTS

Spotfire®
TIBCO Software

临床数字定量病理系统
Vectra Polaris

诊疗



靶点确认
文库建立

抗体筛选
体系优化

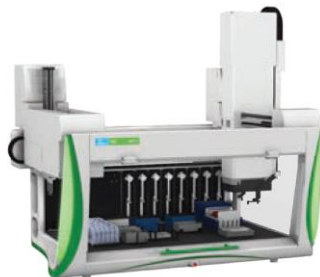
功能验证
安全评价

临床前/临床
测试

工艺开发
生产质控

多标试剂&细胞株
Alpha/Lance/Defia/Lite
Cell line/ Microplate

抗体分析系统
LabChip GXII



生产



缩短
构建时间

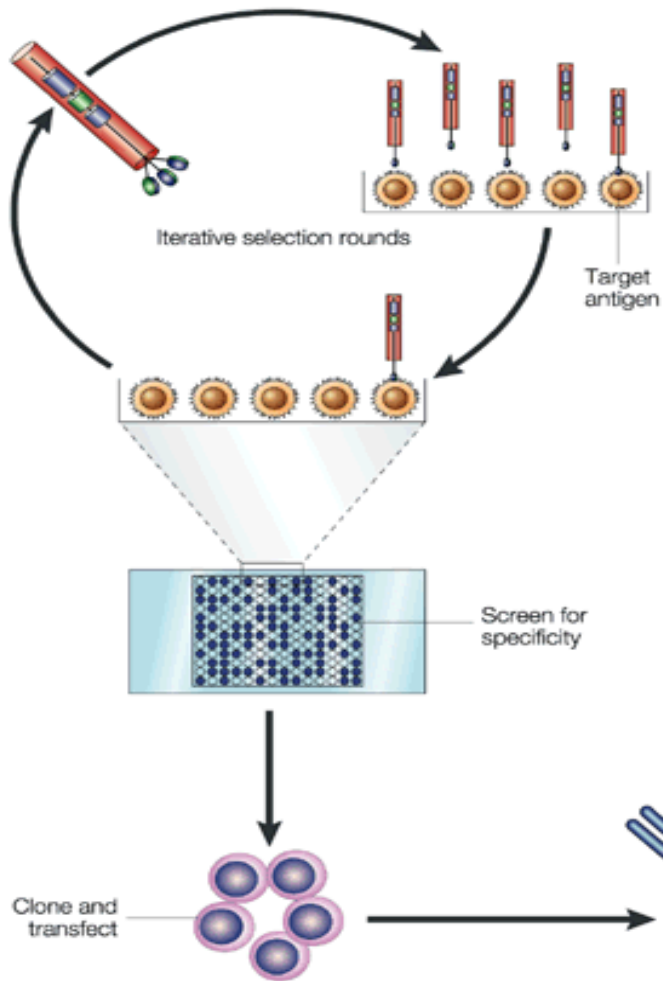
降低
技术成本

自动化样品处理工作站
JANUS Workstation

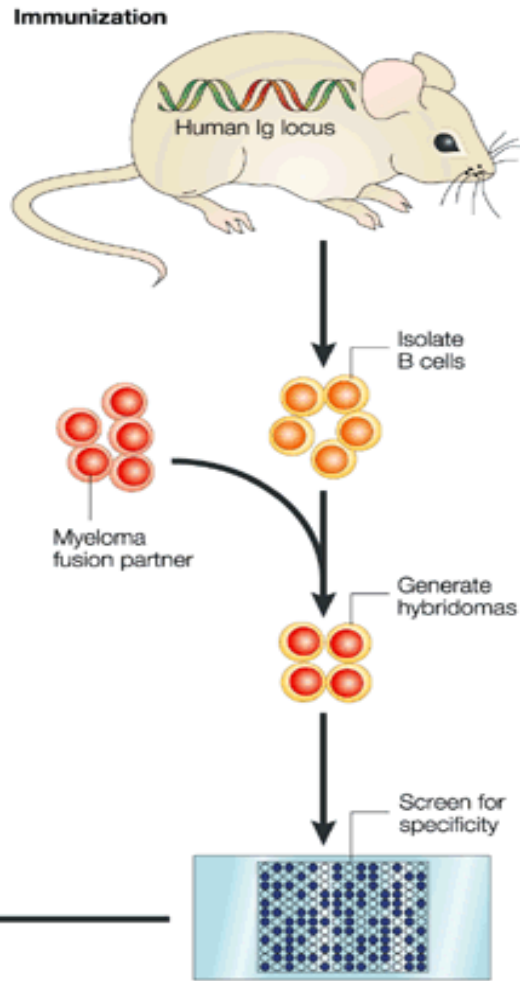
自动化整合解决方案
Cell::Explore

噬菌体文库&杂交瘤抗体筛选技术

a Human antibody library technology



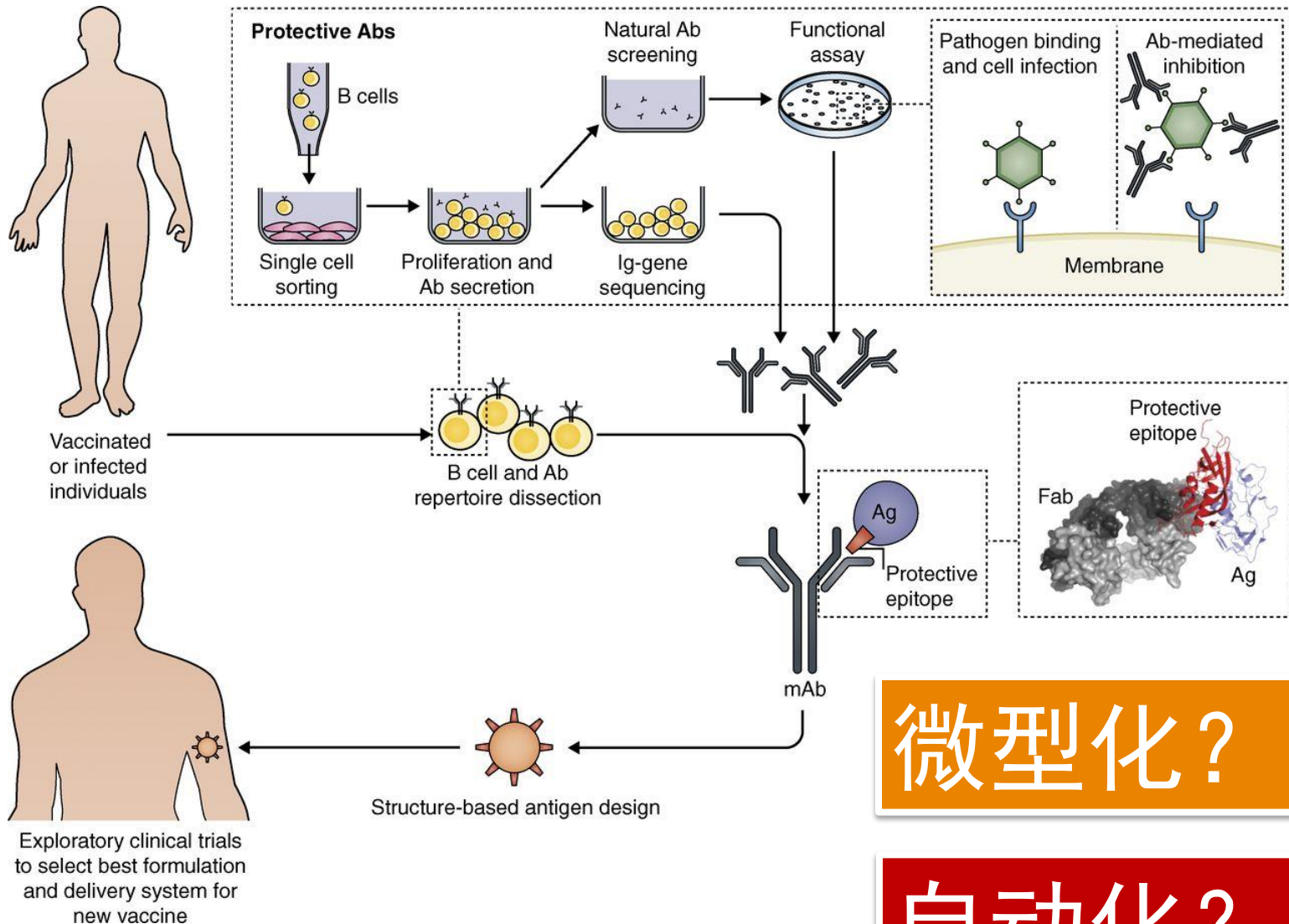
b Transgenic mouse technology



通量?

效率?

人源B细胞抗体/疫苗筛选设计

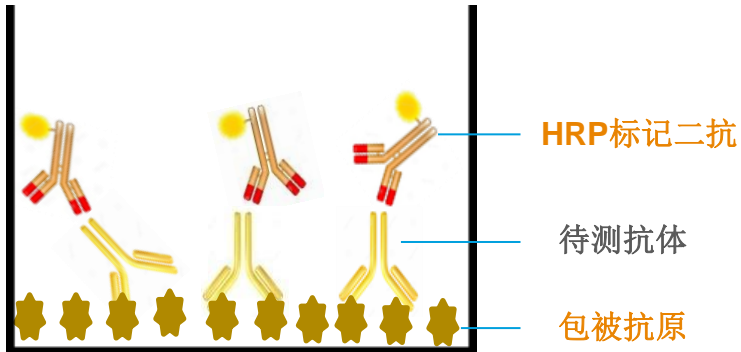


微型化?

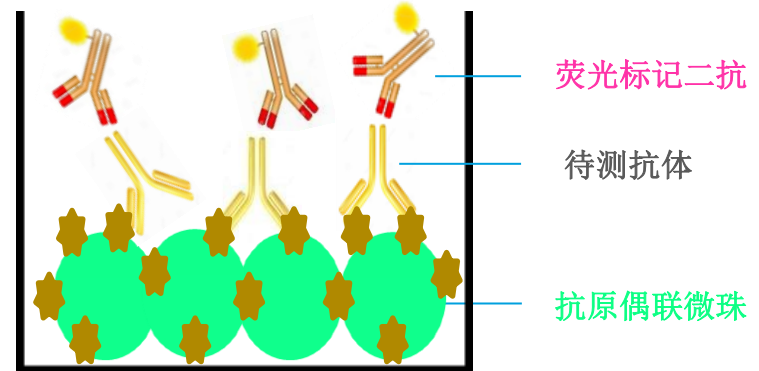
自动化?

抗体&克隆鉴定方法

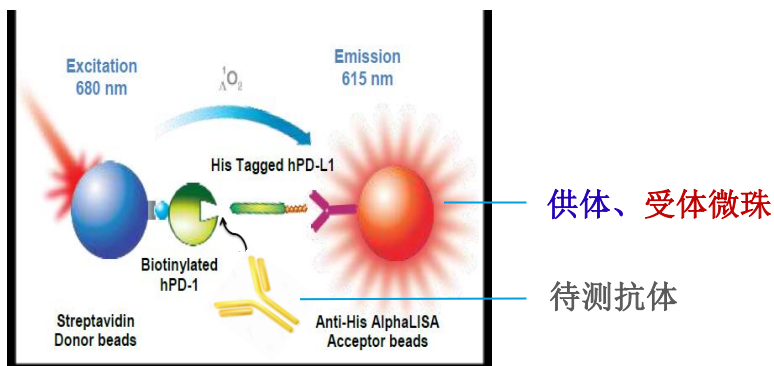
传统ELISA



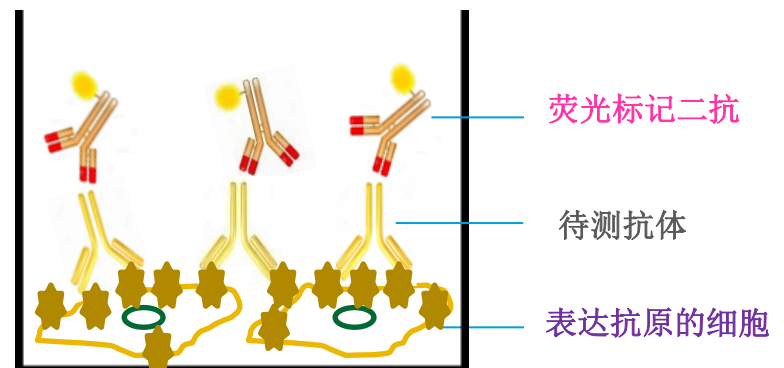
免洗微球成像



免洗高灵敏Alpha



免洗细胞成像



不同抗体筛选方法需要的检测仪器

ELISA/TRF/TR-FRET
(酶标仪)



VICTOR™ Nivo



EnVision®



EnSight™

免洗高灵敏度Alpha
(酶标仪)



EnVision®

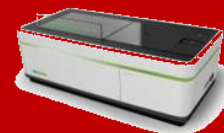


EnSight™

免洗微球成像
(成像酶标仪&HCS)



Operetta® CLS



Opera Phenix™



EnSight™

免洗细胞成像
(成像酶标仪&HCS)



Operetta® CLS



Opera Phenix™



EnSight™

杂交瘤筛选 (vs ELISA)

AlphaScreen Assay Format



AlphaScreen Assay Protocol

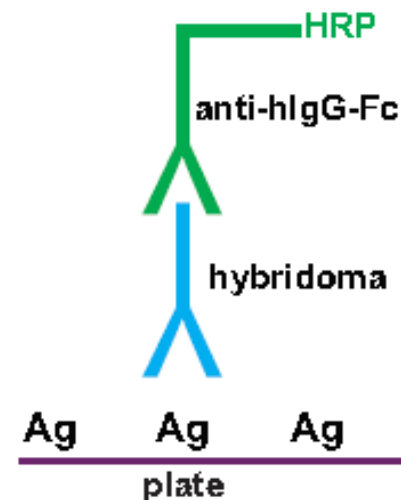
Donor Beads + Acceptor Beads + bio-Ag + Hybridoma Supernatants	
Incubate	2-4 hours
Read Signal	

ELISA Assay Protocol

Antigen Coating Wash x 3	overnight
Blocking Aspirate x 1	2 hours
Hybridoma Supernatants Wash x 3	2 hours
Secondary Antibodies Wash x 3	1 hour
Substrate (TMB) Stop solution	0.5 hour

Sandwich ELISA requires additional step of antibody coating extending time for 2 hours

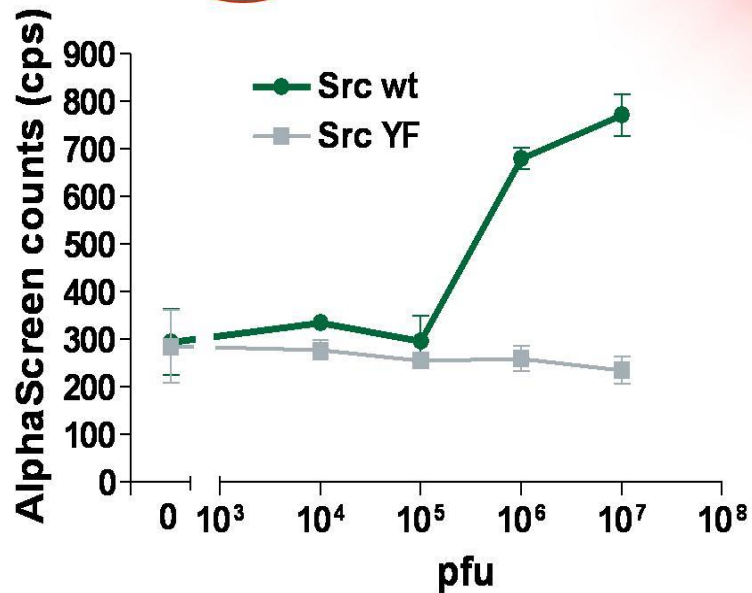
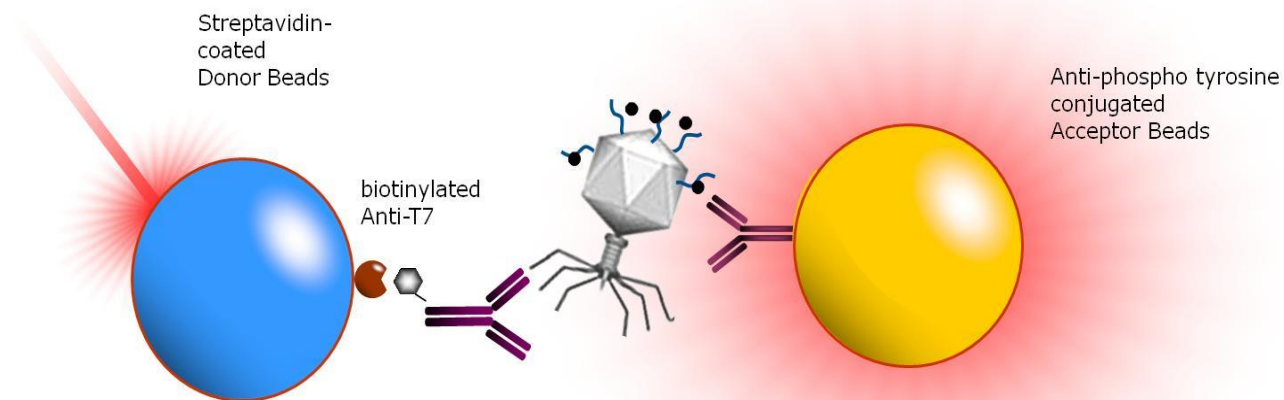
ELISA Assay Format



操作简单

免洗高效

噬菌体文库筛选



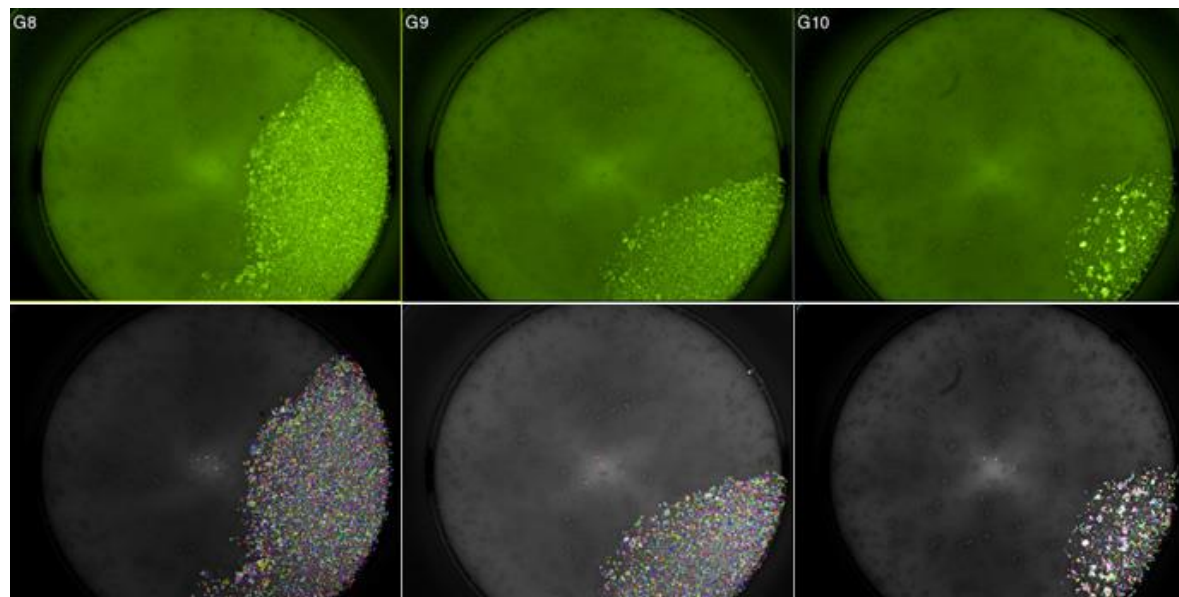
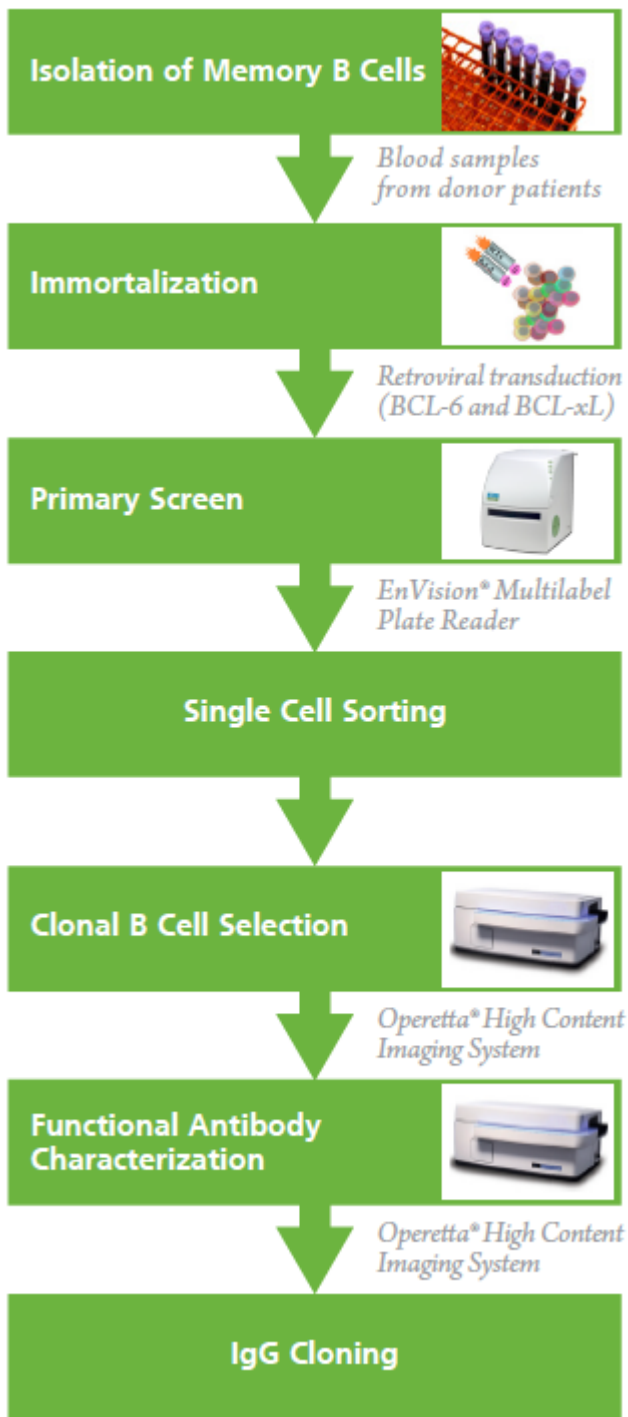
T7-expressed constructs can be specifically phosphorylated by c-Src

This phosphorylation can be detected by anti-phosphotyrosine antibody-coupled beads

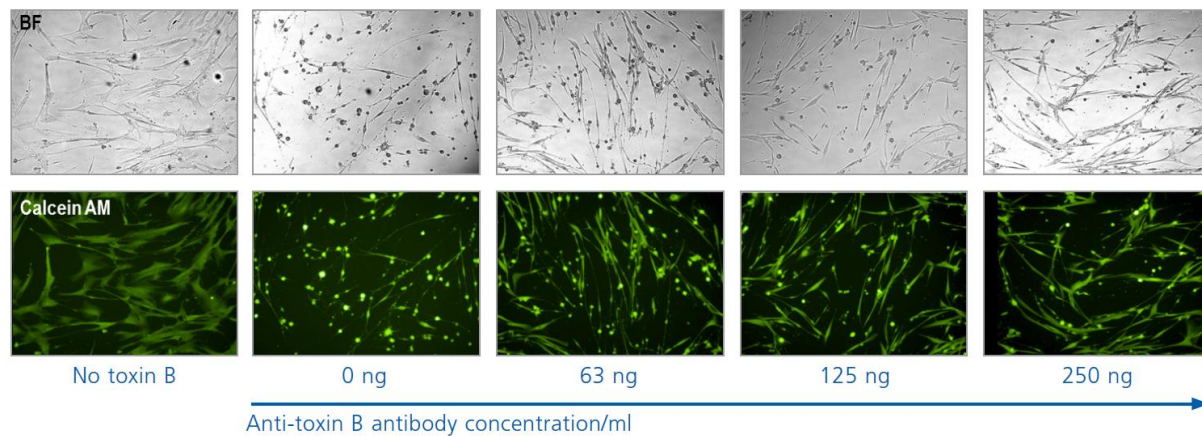
高通量

微型化

人源B细胞抗体筛选及功能研究



B 细胞鉴定



中和性抗体对人源肺纤维细胞 (IMR-90) 形态学功能性验证

抗体筛选自动化整合方案

Protocol

Operator prepares stock solutions of all required reagents (analyte, acceptor beads, biotinylated antibody, donor beads)



JANUS® combines 5 μL of analyte with 10 μL of AlphaLISA anti-analyte acceptor beads and 10 μL of biotinylated anti-analyte antibody



Incubate 60 minutes at 23°C

JANUS® adds 25 μL streptavidin Alpha donor beads to each well in a single step

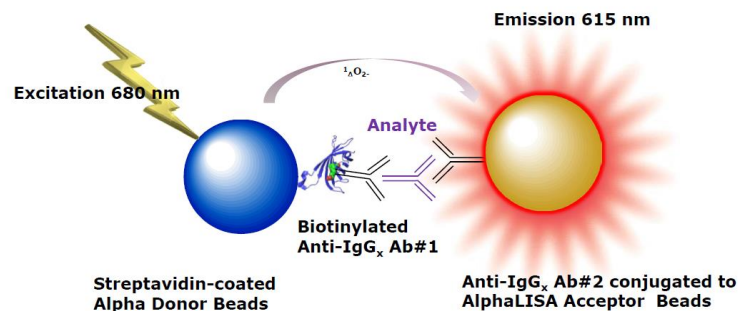


Incubate 30 minutes at 23°C in the dark

Operator removes completed plate and reads using EnVision® or EnSpire® Multimode Plate Reader and analyzes data using GraphPad Prism®



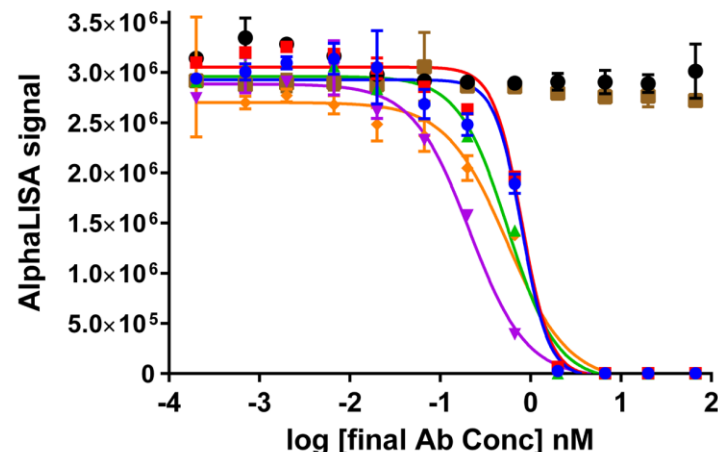
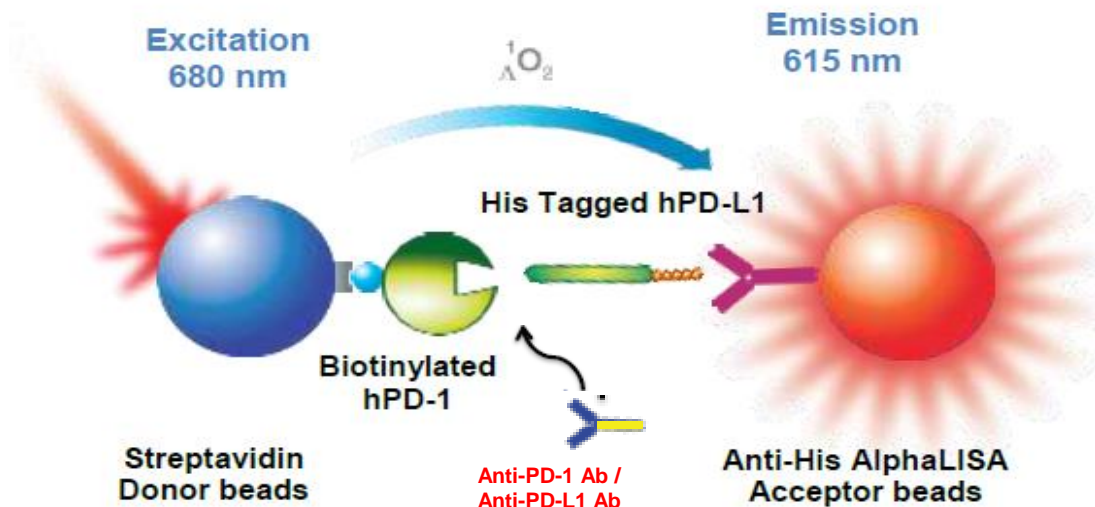
Well	Sample	Intensity	Background	Signal
A1	Control	1000	500	500
A2	Sample 1	1500	500	1000
A3	Sample 2	2000	500	1500
A4	Sample 3	2500	500	2000
A5	Sample 4	3000	500	2500
A6	Sample 5	3500	500	3000
A7	Sample 6	4000	500	3500
A8	Sample 7	4500	500	4000
A9	Sample 8	5000	500	4500
A10	Sample 9	5500	500	5000
A11	Sample 10	6000	500	5500
A12	Sample 11	6500	500	6000
A13	Sample 12	7000	500	6500
A14	Sample 13	7500	500	7000
A15	Sample 14	8000	500	7500
A16	Sample 15	8500	500	8000
A17	Sample 16	9000	500	8500
A18	Sample 17	9500	500	9000
A19	Sample 18	10000	500	9500
A20	Sample 19	10500	500	10000
A21	Sample 20	11000	500	10500
A22	Sample 21	11500	500	11000
A23	Sample 22	12000	500	11500
A24	Sample 23	12500	500	12000
A25	Sample 24	13000	500	12500
A26	Sample 25	13500	500	13000
A27	Sample 26	14000	500	13500
A28	Sample 27	14500	500	14000
A29	Sample 28	15000	500	14500
A30	Sample 29	15500	500	15000
A31	Sample 30	16000	500	15500
A32	Sample 31	16500	500	16000
A33	Sample 32	17000	500	16500
A34	Sample 33	17500	500	17000
A35	Sample 34	18000	500	17500
A36	Sample 35	18500	500	18000
A37	Sample 36	19000	500	18500
A38	Sample 37	19500	500	19000
A39	Sample 38	20000	500	19500
A40	Sample 39	20500	500	20000
A41	Sample 40	21000	500	20500
A42	Sample 41	21500	500	21000
A43	Sample 42	22000	500	21500
A44	Sample 43	22500	500	22000
A45	Sample 44	23000	500	22500
A46	Sample 45	23500	500	23000
A47	Sample 46	24000	500	23500
A48	Sample 47	24500	500	24000
A49	Sample 48	25000	500	24500
A50	Sample 49	25500	500	25000
A51	Sample 50	26000	500	25500
A52	Sample 51	26500	500	26000
A53	Sample 52	27000	500	26500
A54	Sample 53	27500	500	27000
A55	Sample 54	28000	500	27500
A56	Sample 55	28500	500	28000
A57	Sample 56	29000	500	28500
A58	Sample 57	29500	500	29000
A59	Sample 58	30000	500	29500
A60	Sample 59	30500	500	30000
A61	Sample 60	31000	500	30500
A62	Sample 61	31500	500	31000
A63	Sample 62	32000	500	31500
A64	Sample 63	32500	500	32000
A65	Sample 64	33000	500	32500
A66	Sample 65	33500	500	33000
A67	Sample 66	34000	500	33500
A68	Sample 67	34500	500	34000
A69	Sample 68	35000	500	34500
A70	Sample 69	35500	500	35000
A71	Sample 70	36000	500	35500
A72	Sample 71	36500	500	36000
A73	Sample 72	37000	500	36500
A74	Sample 73	37500	500	37000
A75	Sample 74	38000	500	37500
A76	Sample 75	38500	500	38000
A77	Sample 76	39000	500	38500
A78	Sample 77	39500	500	39000
A79	Sample 78	40000	500	39500
A80	Sample 79	40500	500	40000
A81	Sample 80	41000	500	40500
A82	Sample 81	41500	500	41000
A83	Sample 82	42000	500	41500
A84	Sample 83	42500	500	42000
A85	Sample 84	43000	500	42500
A86	Sample 85	43500	500	43000
A87	Sample 86	44000	500	43500
A88	Sample 87	44500	500	44000
A89	Sample 88	45000	500	44500
A90	Sample 89	45500	500	45000
A91	Sample 90	46000	500	45500
A92	Sample 91	46500	500	46000
A93	Sample 92	47000	500	46500
A94	Sample 93	47500	500	47000
A95	Sample 94	48000	500	47500
A96	Sample 95	48500	500	48000
A97	Sample 96	49000	500	48500
A98	Sample 97	49500	500	49000
A99	Sample 98	50000	500	49500
A100	Sample 99	50500	500	50000



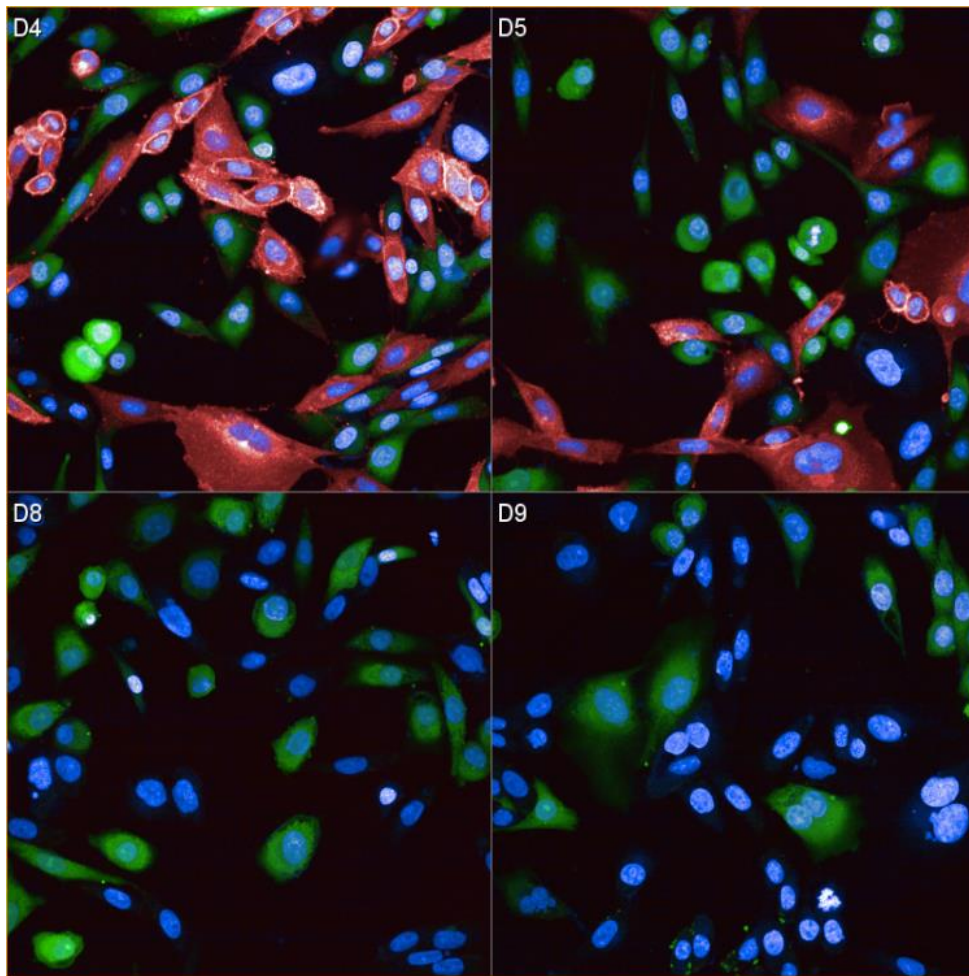
分子水平：PD-1/PD-L1免疫检验点阻断剂

企业	美施贵宝 (BMS)	默沙东 (MSD)	罗氏 (Roche)	默克/辉瑞 (Merck/Pfizer)	阿斯利康 (AstraZeneca)
靶点	PD-1	PD-1	PD-L1	PD-L1	PD-L1
商品名	Opdivo	Keytruda	Tecentriq	Bavencin	Imfinzi
通用名	Nivolumab	Pembrolizumab	Atezolizumab	Avelumab	DuValumab
获批适应症	转移性黑色素瘤 非小细胞肺癌 晚期肾细胞癌 霍奇金淋巴瘤 头颈癌 尿路上皮癌	晚期黑色素瘤 转移性黑色素瘤 非小细胞肺癌 头颈癌	尿路上皮癌 非小细胞肺癌	Merkel 细胞癌	尿路上皮癌
FDA批准日期	2014.12.22 2014.07.07 (日本)	2014.09.04	2016.05.18	2017.03.23	2017.05.01

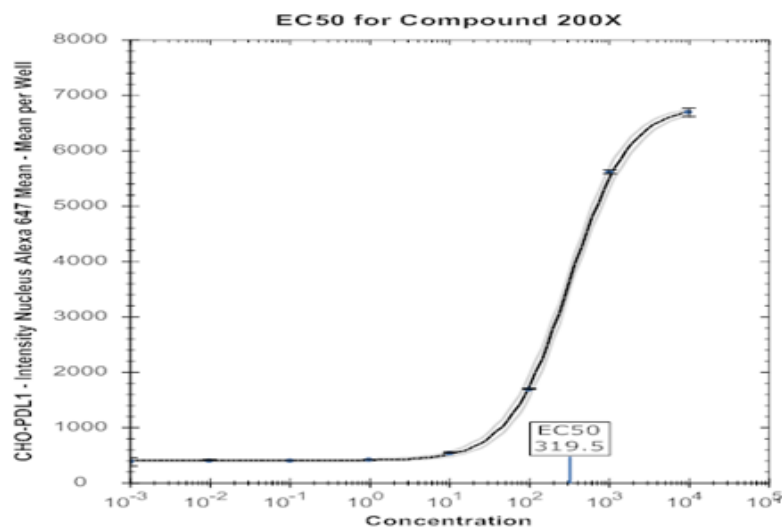
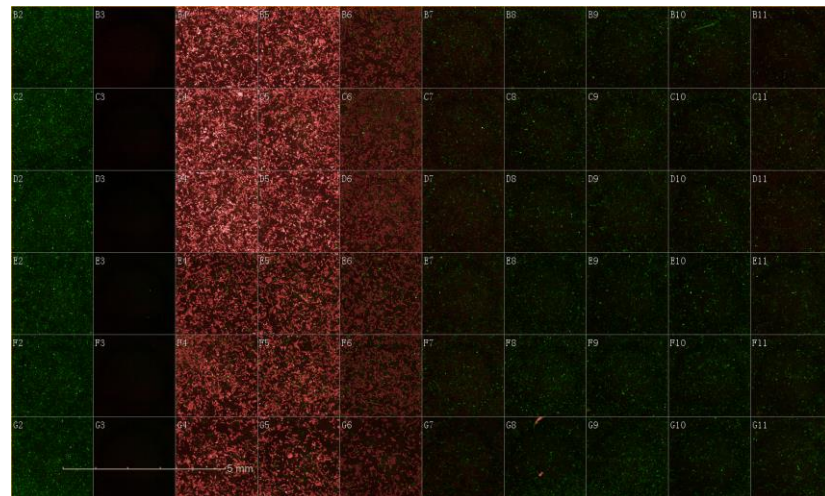
- Nivolumab-IgG4 (Anti-PD-1)
- Nivolumab-IgG1 (Anti-PD-1)
- ▲ Anti-PD-1 (from Biolegend)
- ▼ Atezolizumab (Anti-PD-L1)
- ◆ Anti-PD-L1 (from ACRO)
- IgG1-Control
- IgG4-Control



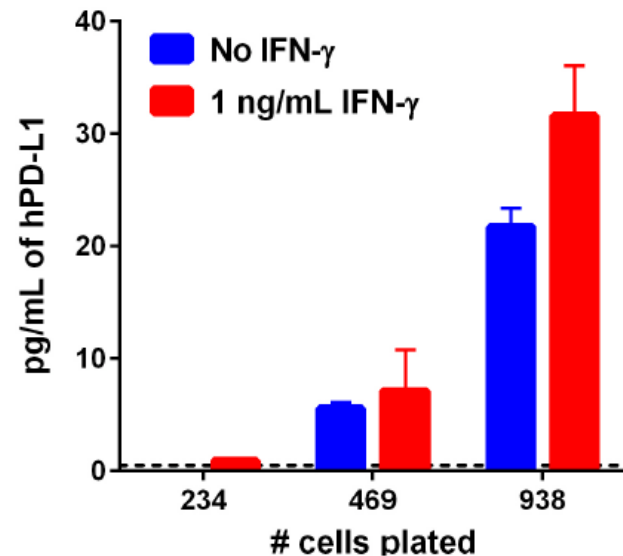
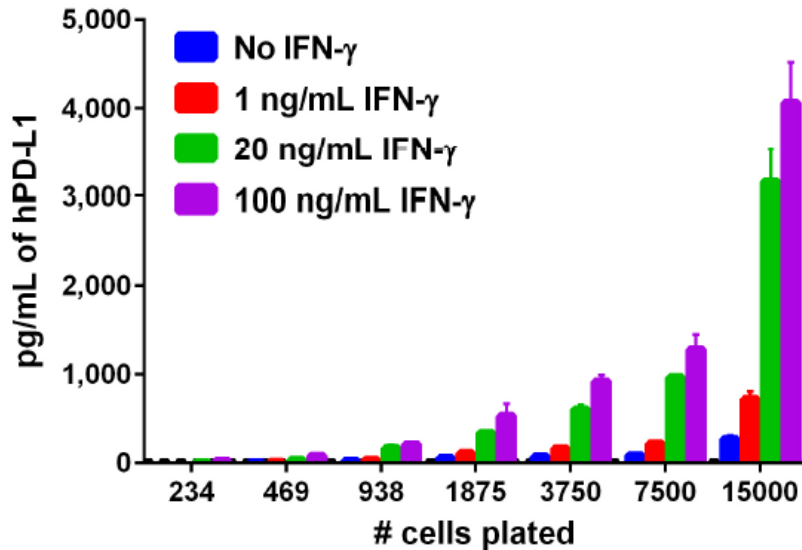
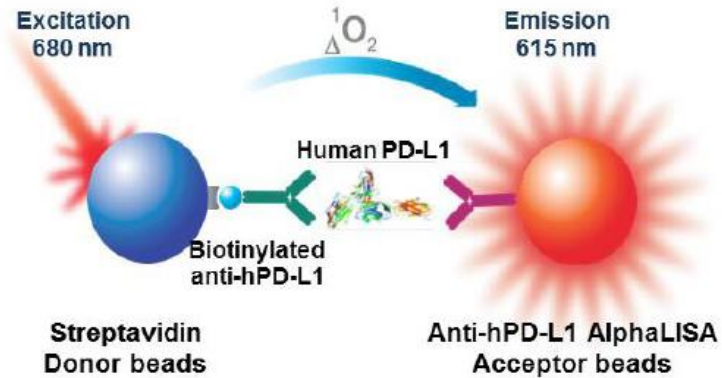
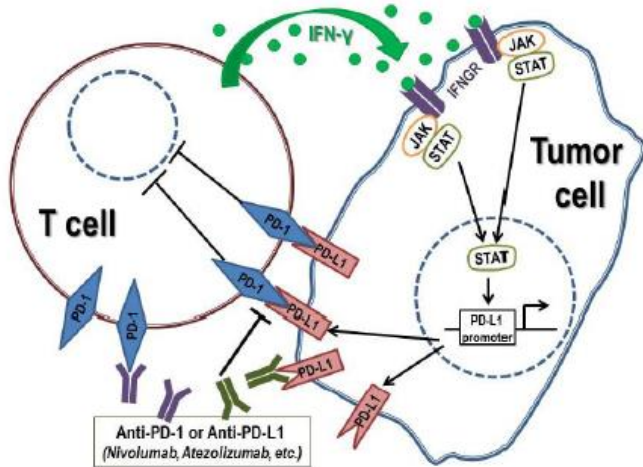
细胞水平抗体结合实验: PD-L1 抗体筛选



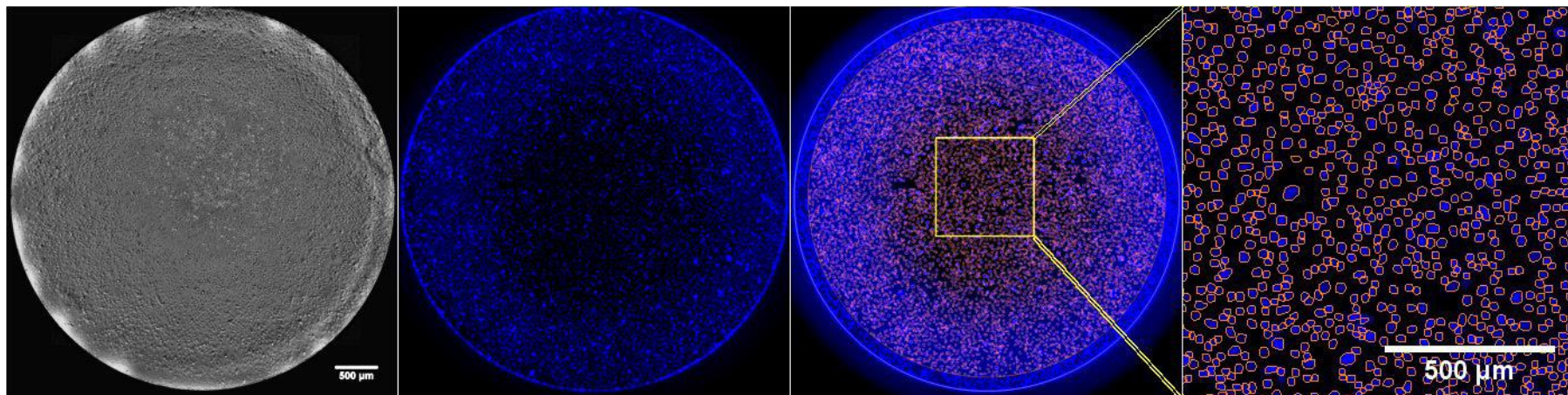
CHO-K1: Cell Track Green; CHO-K1-PDL1: No dye
1st Ab: Mouse-anti PDL1 antibody; 2st: Goat anti-mouse IgG-AlexaFluor647



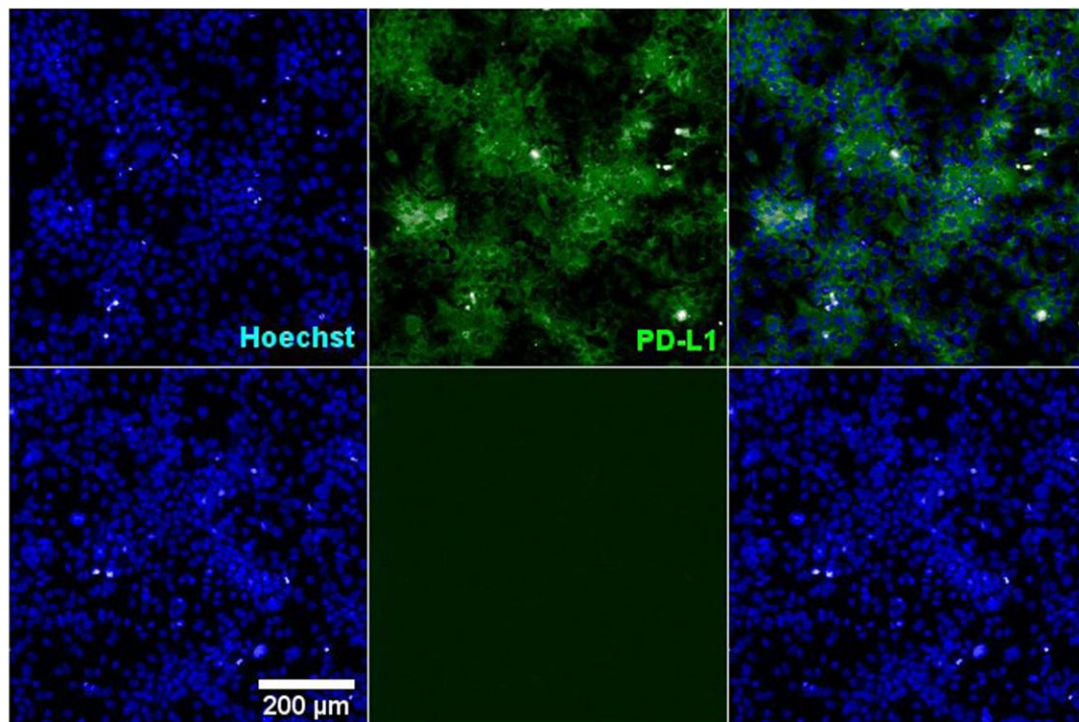
PD-L1 蛋白表达量检测及信号通路研究



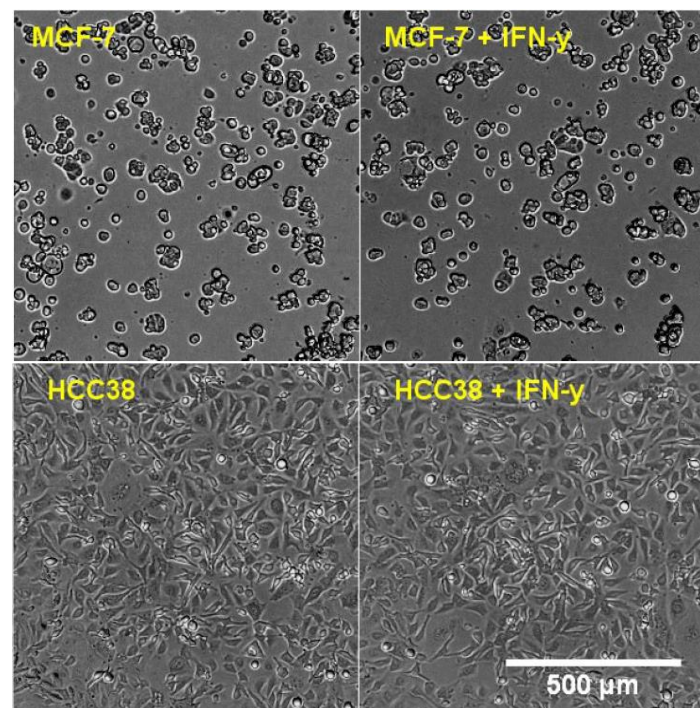
整孔显微成像系统进行细胞水平PD-L1表达量分析



IFN-γ处理组



无IFN-γ对照组



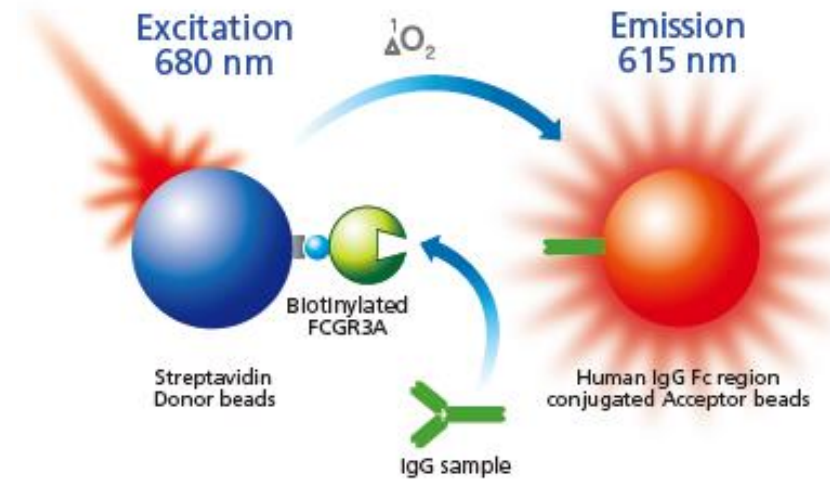
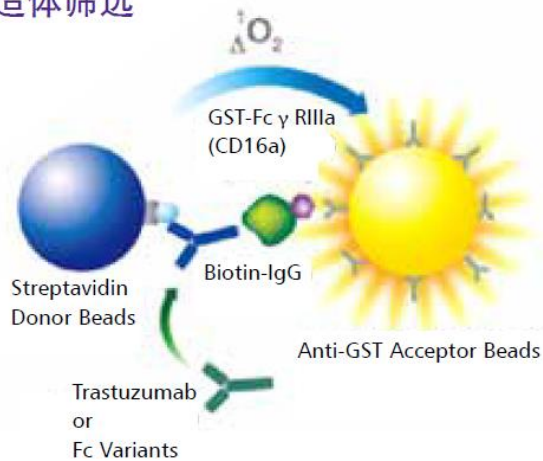
蓝色: Hoechst标记细胞核

绿色: 荧光素标记PD-L1蛋白

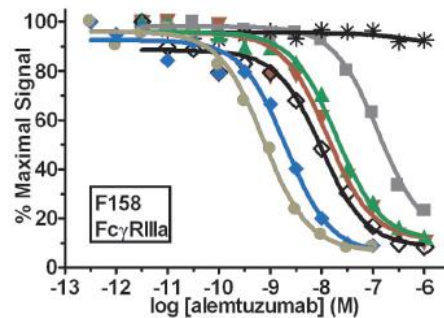
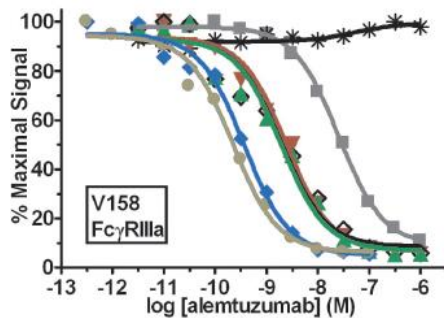
蓝色+绿色叠加

Trastuzumab Fc区改造优化ADCC活性

Fc区域改造体筛选



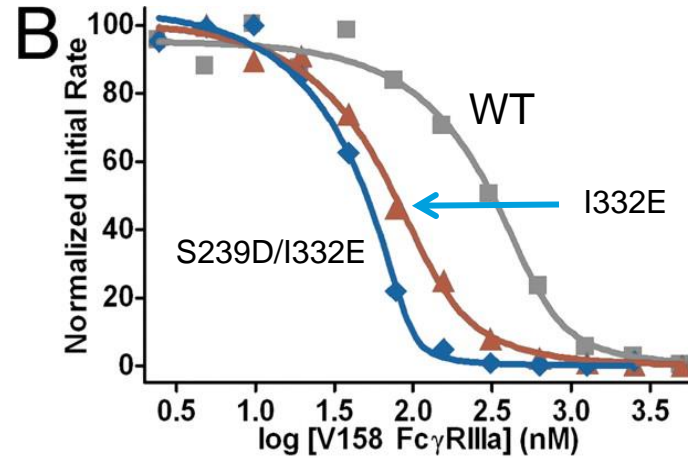
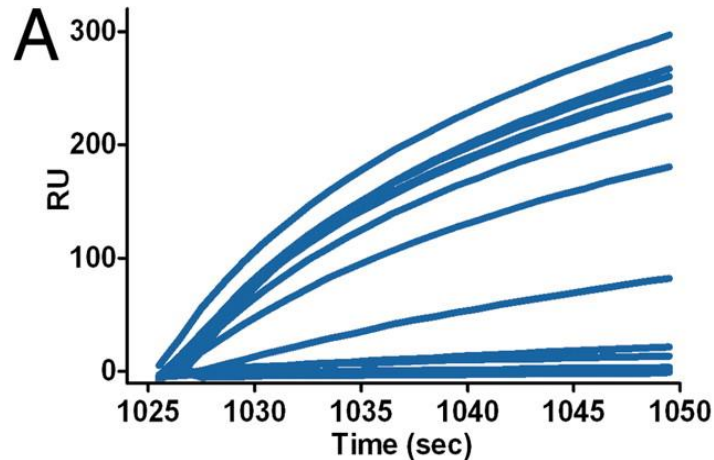
Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, Chan C, Chung HS, Eivazi A, Yoder SC, Vielmetter J, Carmichael DF, Hayes RJ, Dahiyat BI. Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci U S A. 2006 Mar 14; 103 (11) : 4005-1 Xencor-USA



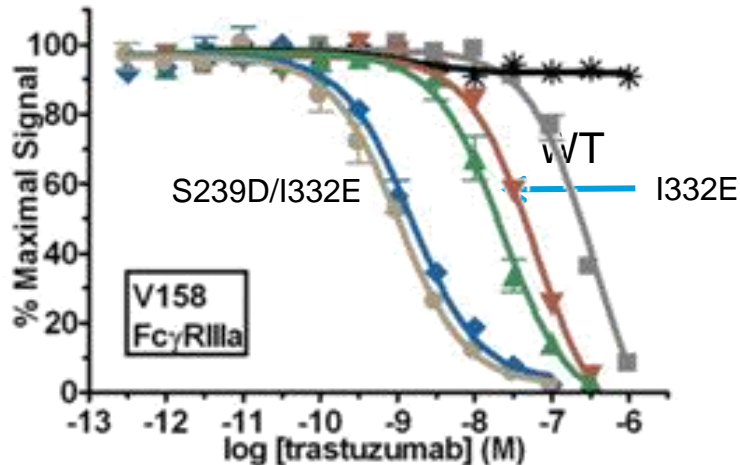
产品名称	数量 (well)	货号
AlphaLISA FCGR3A (176Val) Binding Kit	100	AL347HV
AlphaLISA FCGR3A (176Val) Binding Kit	500	AL347C
AlphaLISA FCGR3A (176Val) Binding Kit	5,000	AL347F
Biotinylated Human FCGR3A (176Phe)	1.5 μ g	AL347S
AlphaLISA FCGR3A (176Val) Binding Kit	100	AL348HV
AlphaLISA FCGR3A (176Val) Binding Kit	500	AL348C
AlphaLISA FCGR3A (176Val) Binding Kit	5,000	AL348F
Biotinylated Human FCGR3A (176Val)	1.5 μ g	AL348S

Trastuzumab Fc区改造对FcγRIIIa亲和力评价

Label Free (SPR)



Alpha

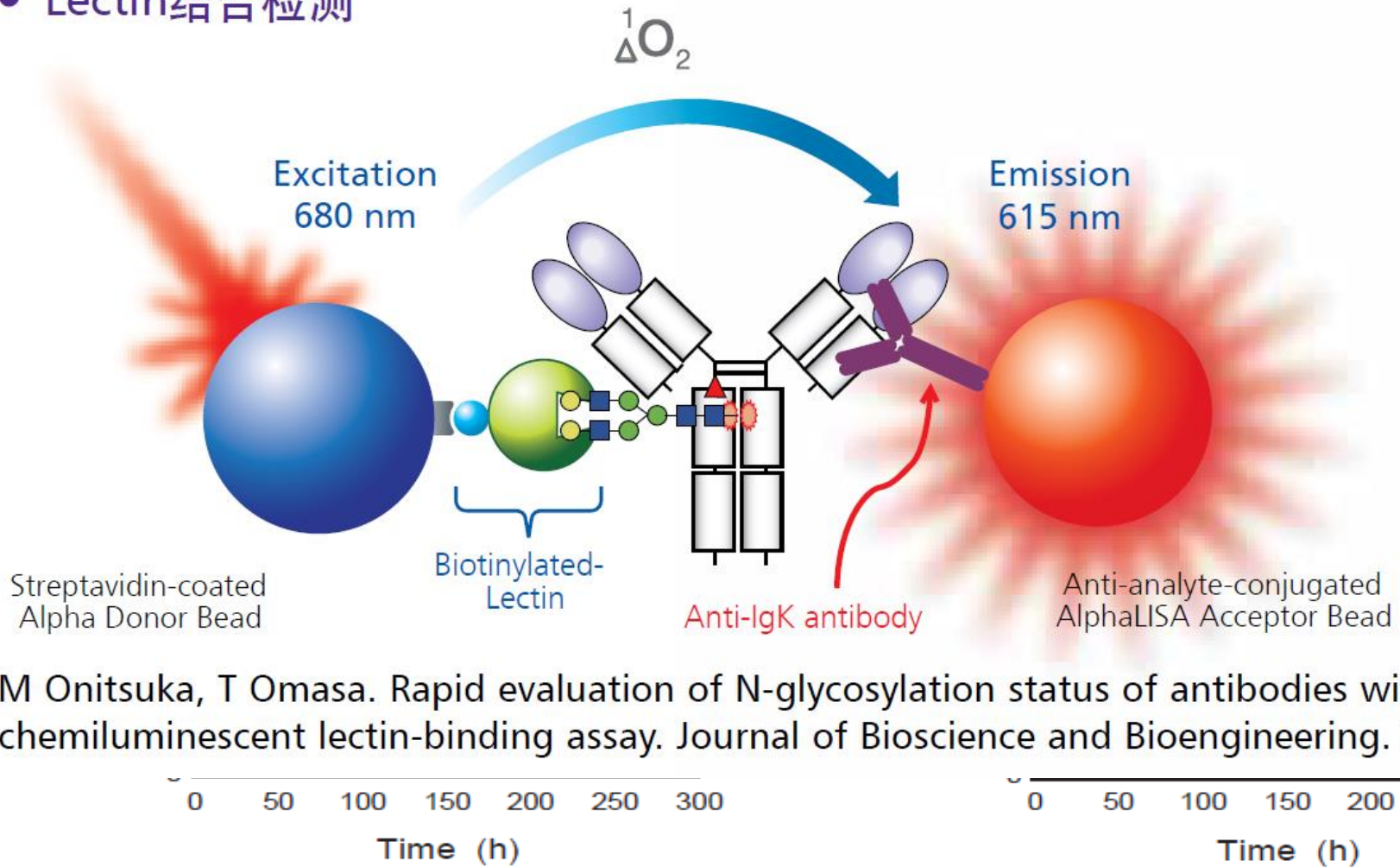


**Alpha and Label-Free
Order of Potency
Measurements results
are Similar**

Alpha Data Consistent with “Gold Standard” BiaCore

抗体糖基化检测

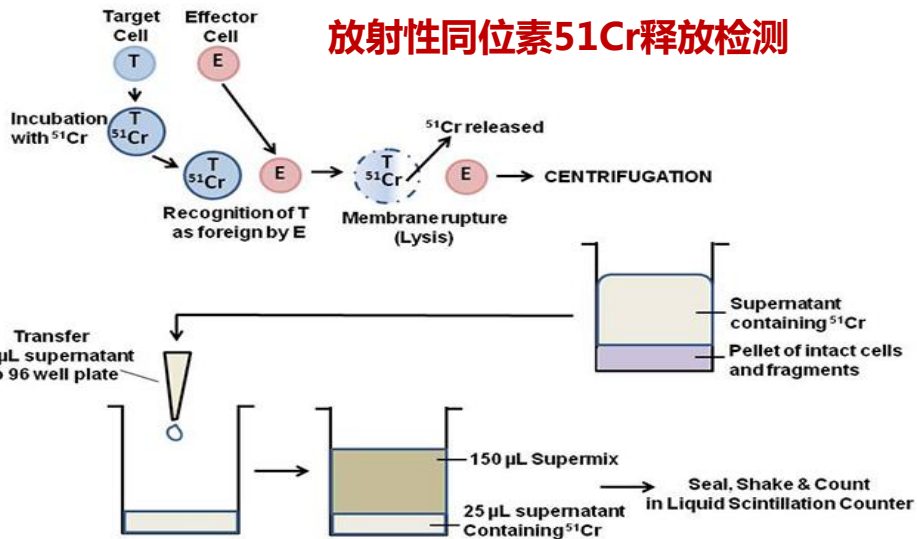
- Lectin结合检测



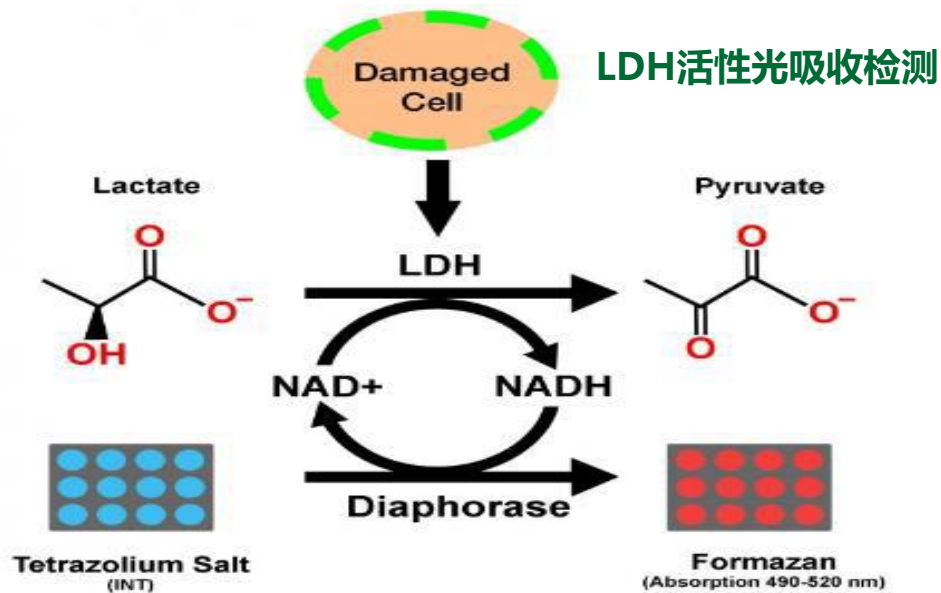
M Onitsuka, T Omasa. Rapid evaluation of N-glycosylation status of antibodies with chemiluminescent lectin-binding assay. *Journal of Bioscience and Bioengineering*. 2014 Dec.

免疫细胞杀伤肿瘤活性评价

放射性同位素⁵¹Cr释放检测

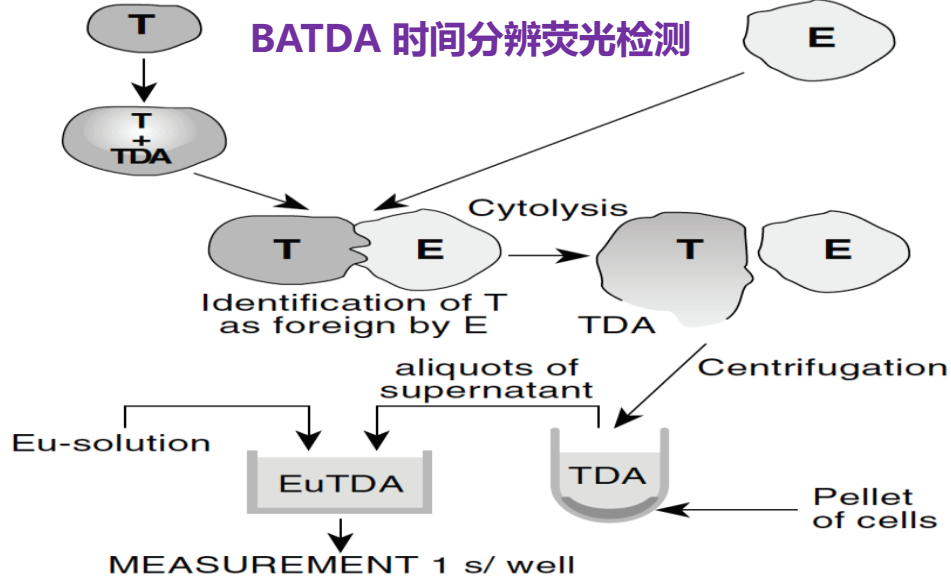


LDH活性光吸收检测

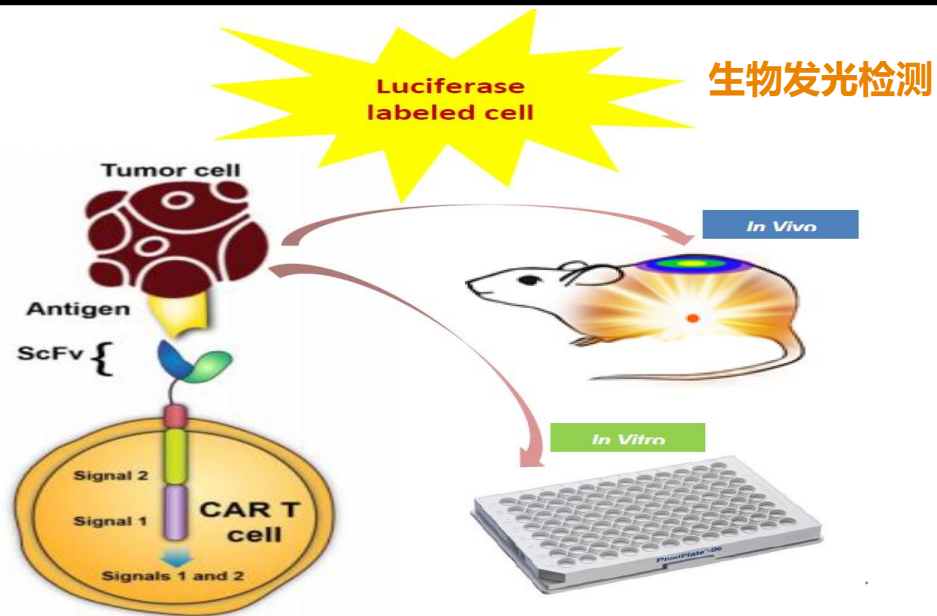


Target cells (T) Effector cells (E)

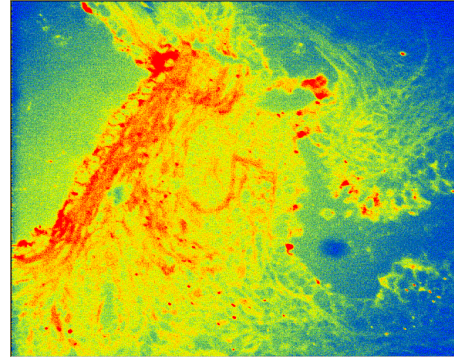
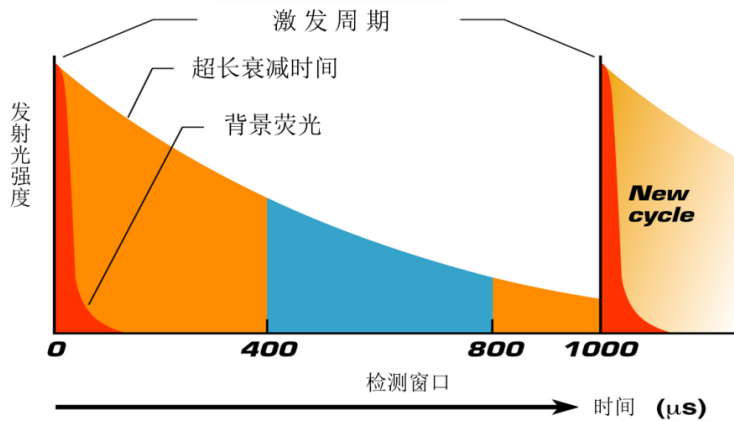
BATDA 时间分辨荧光检测



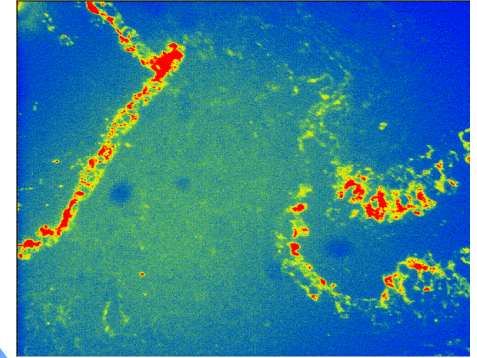
生物发光检测



新一代分子免疫检测：时间分辨荧光（TRF/DELFI A）

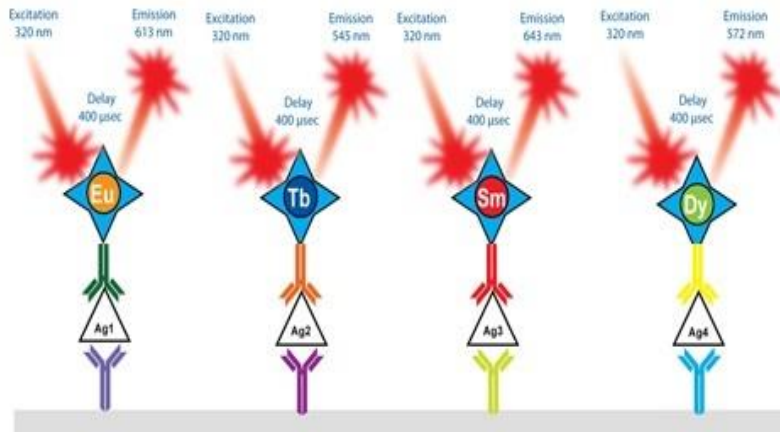


Low S/N



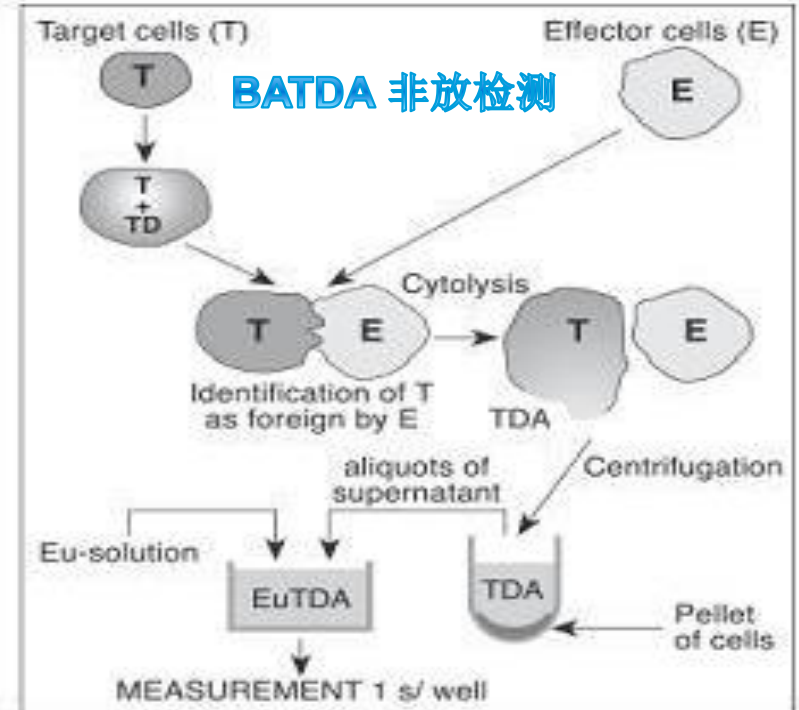
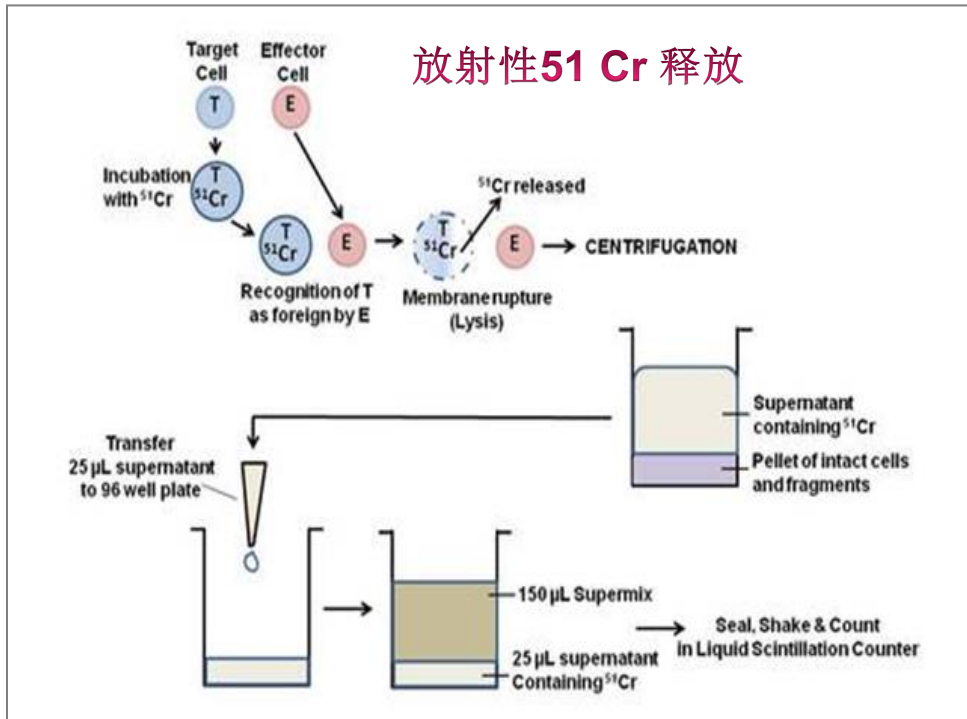
High S/N

Delay



- Applications:** Clinical screening • Cord blood banking • Cytogenetics
- Diabetes • Hemoglobinopathies • Hepatitis B and HIV testing
 - Newborn screening for metabolic disorders • Oncology • Pre-eclampsia and preterm birth • Prenatal risk assessment for trisomy disorders
 - Reproductive hormones and infertility • Thyroid diseases

免疫细胞杀伤活性 (DELFIA BATDA Cytotoxicity)



DELFIA BATDA Cytotoxicity Kit

Features

High sensitivity

Only non-rad, non-enzymatic assay mimicking ⁵¹Cr release

Based on TRF

Fast release of label from cytolized cells

Ability to freeze TDA loaded cells

More efficient and mild labelling than for ⁵¹Cr

Benefits

Less cells required

Non-radioactive technology, eliminating radioactive waste disposal, associated stringent procedures and costs

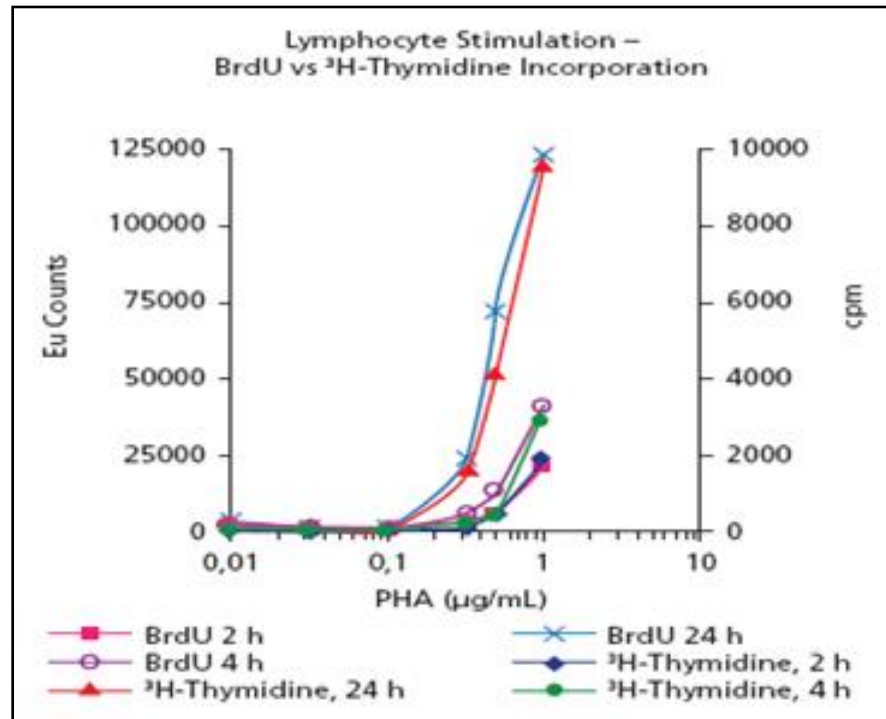
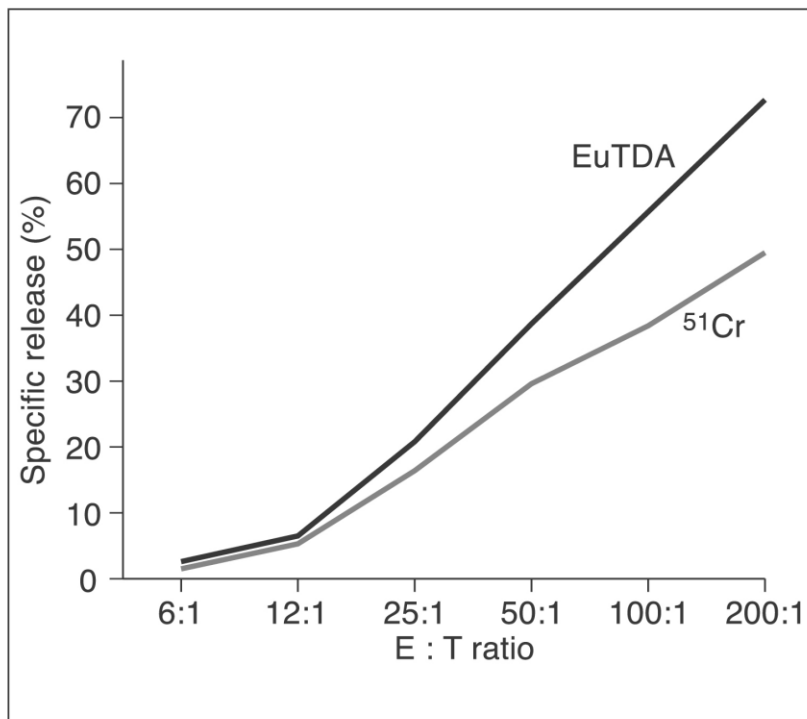
Stable signals can be measured over-and-over or the next day --- flexibility

8 hr ⁵¹Cr release test can be shortened to 2-4 hrs

Flexibility

Shorter labeling time

DELFI A与传统放射性同位素实验结果对比



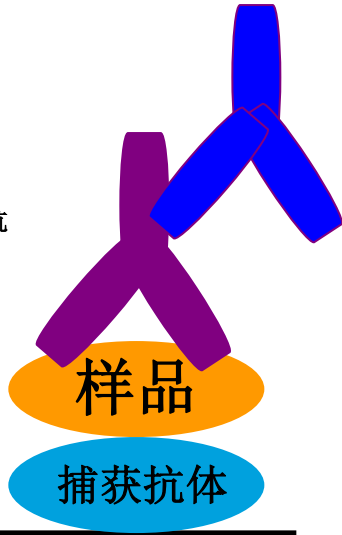
DELFI A比同位素方法具有更高的灵敏度、安全性及适用性

ELISA (光吸收) vs DELFIA (TRF)

TMB A450

HRP

鼠源一抗

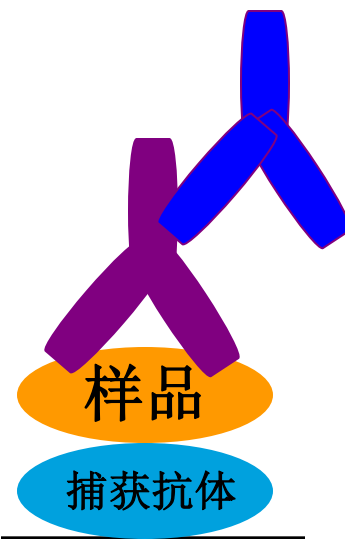


解离增强液

Eu

Eu

鼠源一抗



- ✓ 高灵敏度
- ✓ 高信噪比
- ✓ 线性范围宽
- ✓ 信号稳定
- ✓ 数据可靠

Eu Labeled Antibodies

- Anti-mouse
- Anti-human
- Anti-rabbit
- Anti-HA
- Anti-6His
- Anti-GST
- Anti-c-myc

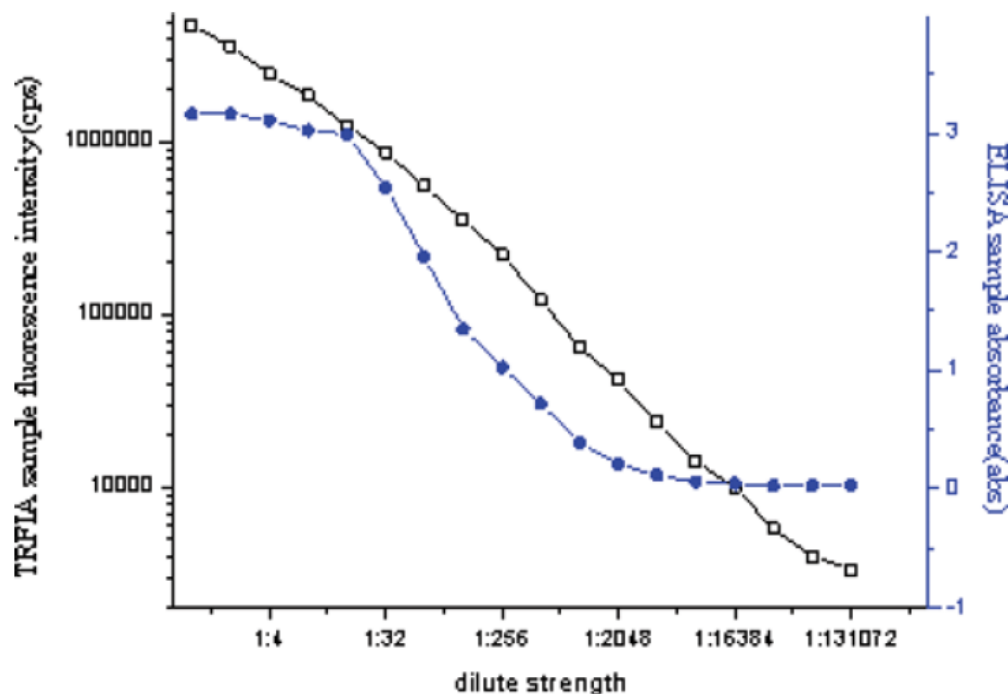
Eu, Tb, and Sm labeled
Streptavidin

DETECTION OF HEPATITIS B VIRUS PRES1 ANTIGEN USING A TIME-RESOLVED FLUOROIMMUNOASSAY

Zhigang Hu,¹ Mei Li,¹ Biao Huang,² Jie Liu,¹ Lei Yu,¹ and Guoqian Chen¹

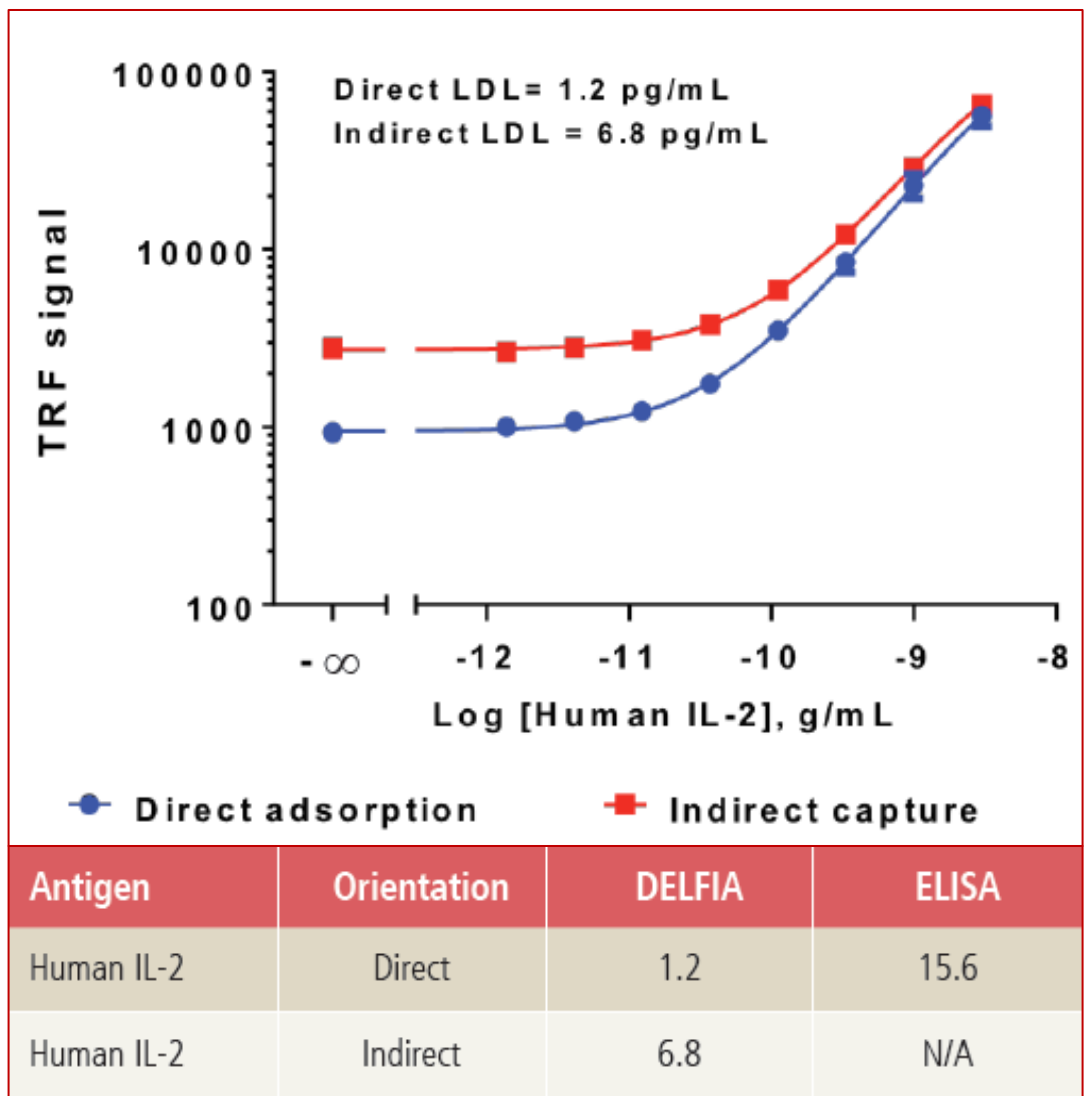
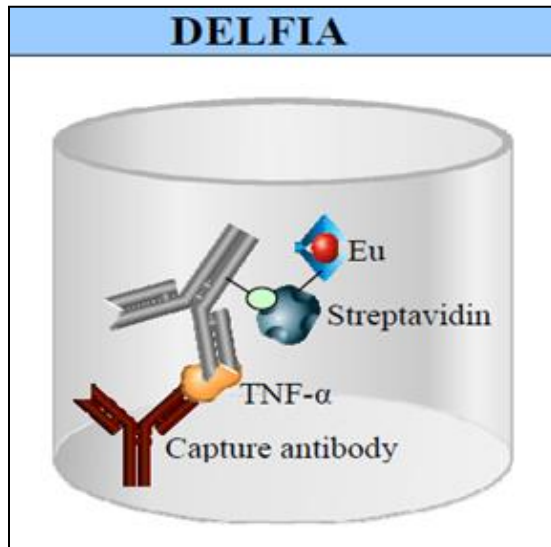
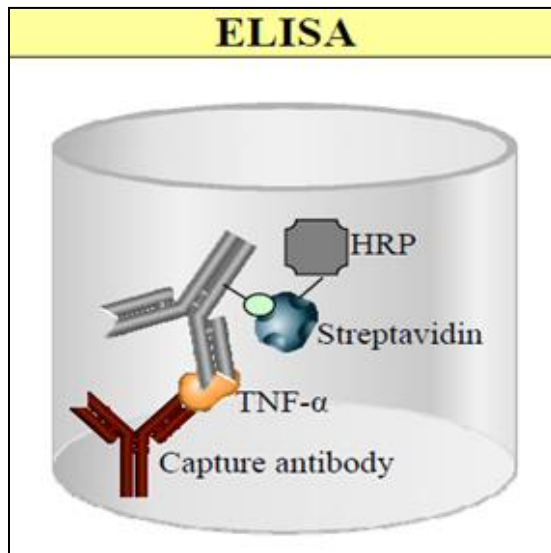
¹Wuxi People's Hospital, Wuxi, China

²Jiangsu Institute of Nuclear Medicine, Wuxi, China



无锡人民医院Chen Guoqian教授等人，使用TRFIA和ELISA两种方法，检测病人血样中新颖的诊断标记物HBV PreS1表面抗原含量，通过平行对比，TRFIA在0.01 ng/ml极低样品浓度下，尚有很好的分辨率，比ELISA的检测灵敏度提高超过16倍，动态检测范围提高超过三个数量级，且对病人血样有更好的抗干扰能力和稳定性。

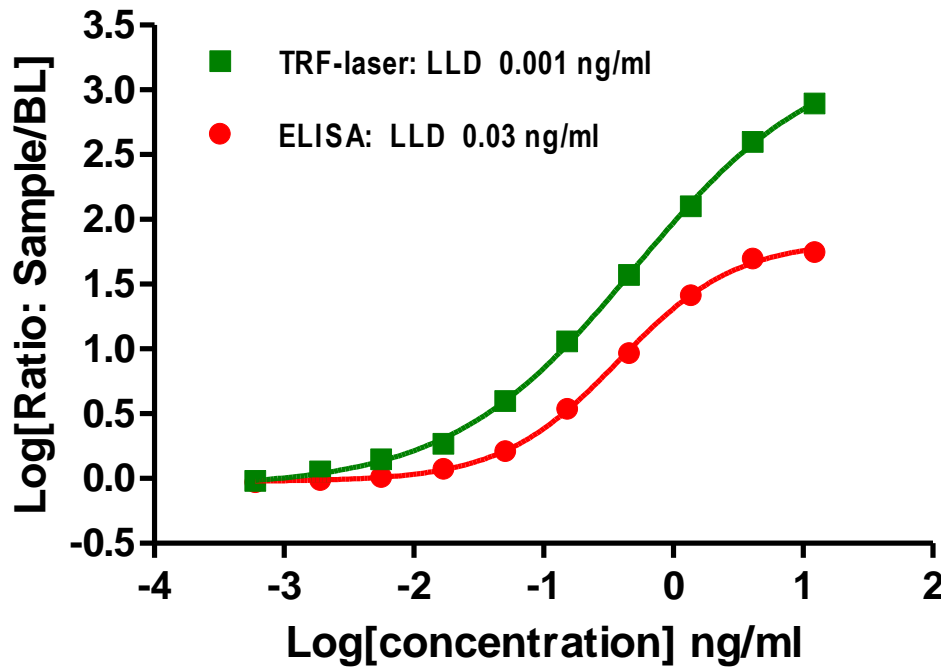
Convert ELISA to DELFIA in one step



行正术奇 百年成长



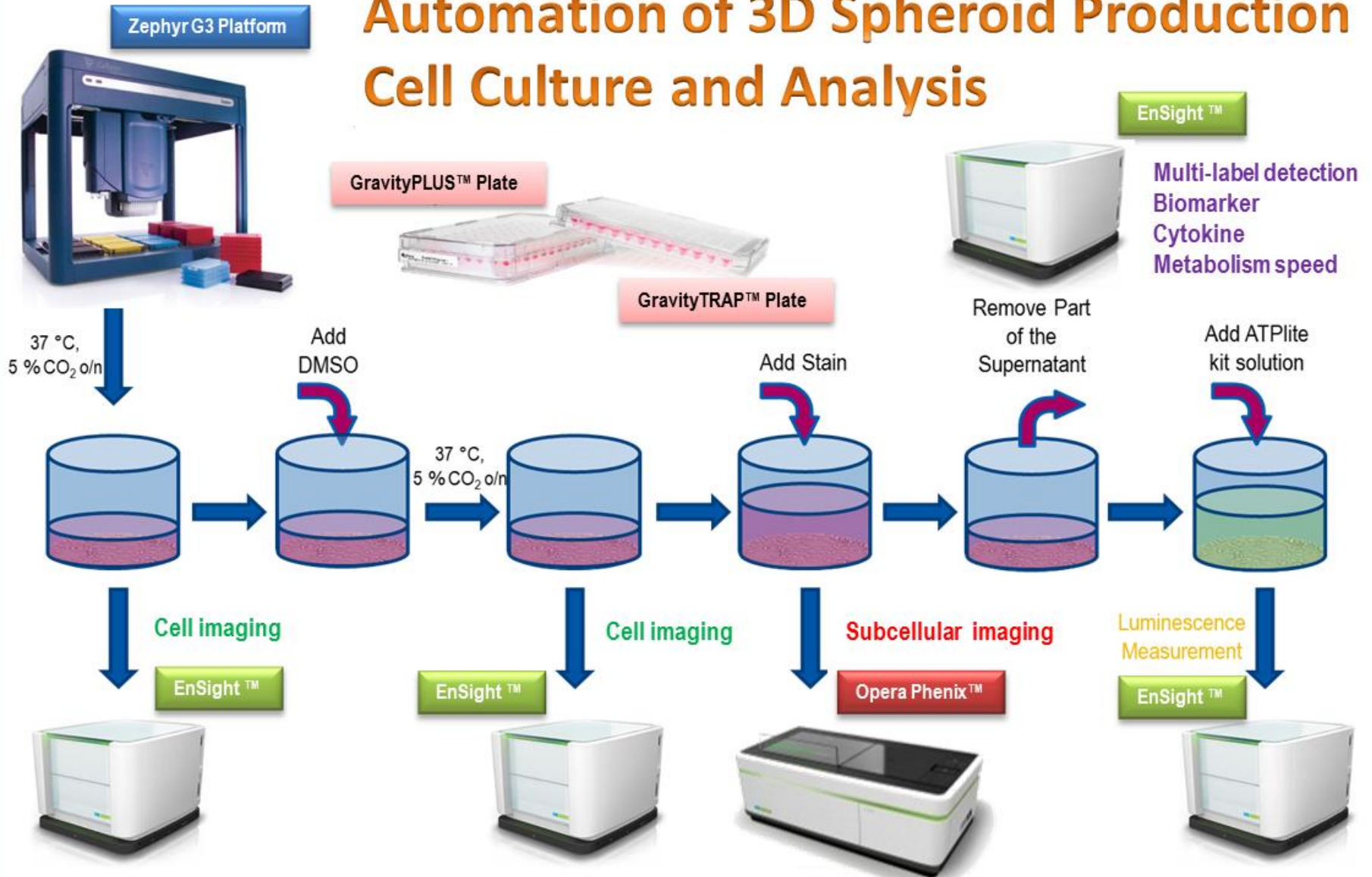
ELISA vs DELFIA



Interpolated by 4 parameters
Standard Curve (ng / ml)

Sample	ELISA	DELFLIA
LLD	0.0303	0.0010
12.3000	8.5671	12.2323
4.1000	5.2834	4.3314
1.3667	1.3667	1.3208
0.4556	0.4354	0.4581
0.1519	0.1577	0.1671
0.0506	0.0499	0.0502
0.0169	0.0183	0.0152
0.0056	0.0061	0.0060
0.0019	0.0018	0.0040
0.0006	/	0.0011

Automation of 3D Spheroid Production Cell Culture and Analysis

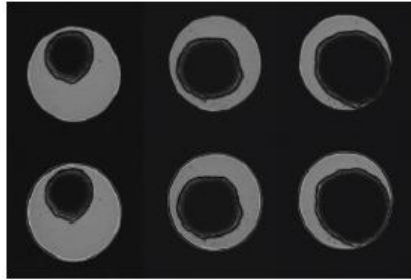


Cell: Explore 客户定制整合系统

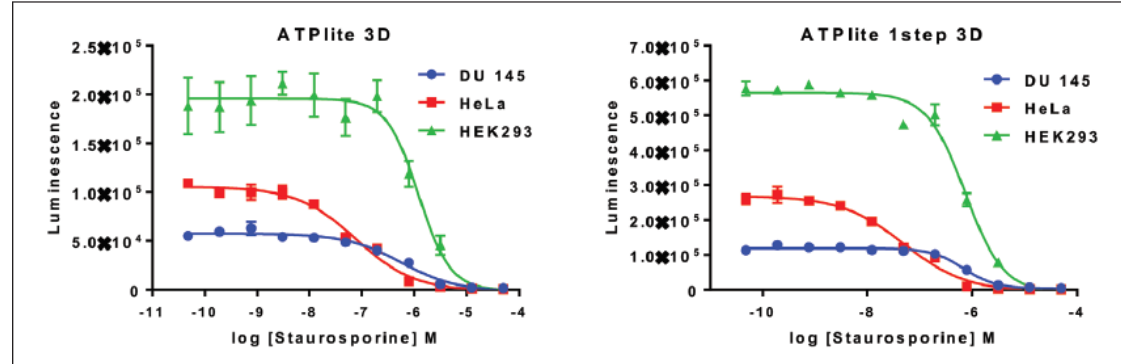
3D Spheroid Cell Culture and Analysis



InSphero GravityPLUS Hanging-Drop System



Spheroids imaged in GravityTRAP plate on the EnSight multimode plate reader and measured to determine their size.



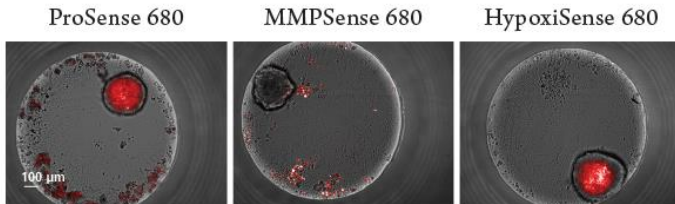
Cytotoxicity study of DU 145, HeLa, and HEK293 spheroids treated with staurosporine, using CellCarrier™ Spheroid ULA and InSphero GravityTRAP microplates, ATPlite 3D and ATPlite 1step 3D assays, and EnSight plate reader.⁵

From HTS To HCS

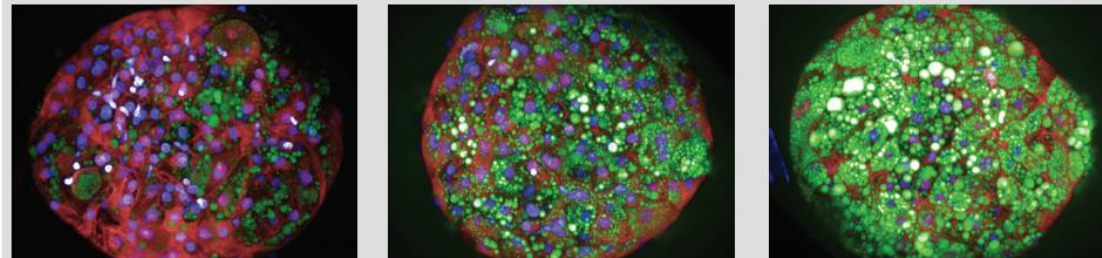


PHENOTYPIC HIGH-CONTENT ASSAY TO STUDY STEATOSIS IN 3D LIVER SPHEROIDS

Several drugs are associated with the potential for drug-induced hepatic steatosis. In this example, lipid accumulation is analyzed in InSphero's 3D InSight™ Human Liver Microtissues labeled with Hoechst (blue, nucleus), CellMask Deep Red (red, membranes), and NileRed (green, lipid droplets). Stack imaging was performed on the Opera Phenix system using a 20xW objective.



Overlay of brightfield and fluorescence images of 3D InSight™ Human Tumor Microtissues stained with ProSense 680, MMPsense 680, and Hypoxisense 680 results in characteristic staining patterns.⁷

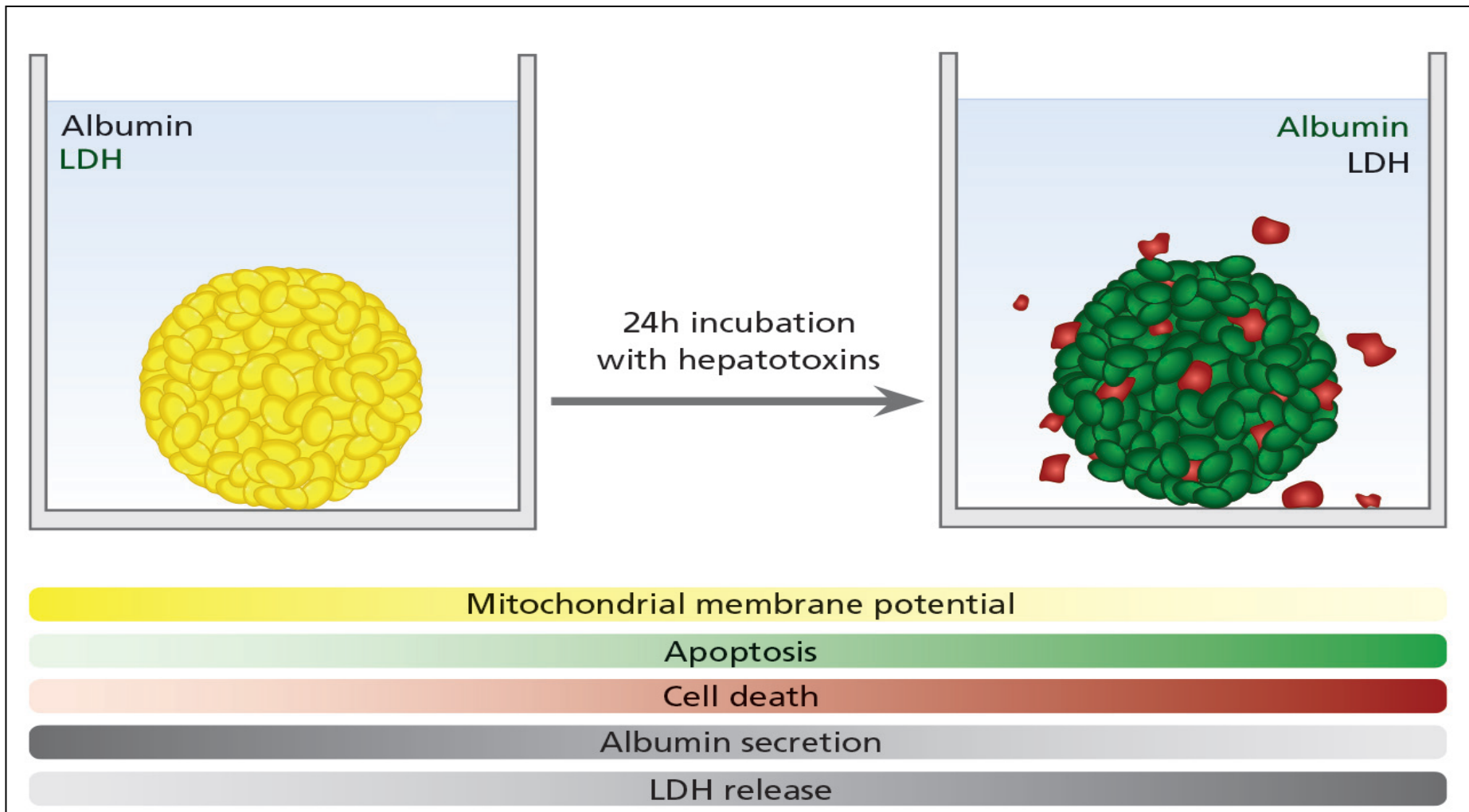


Control

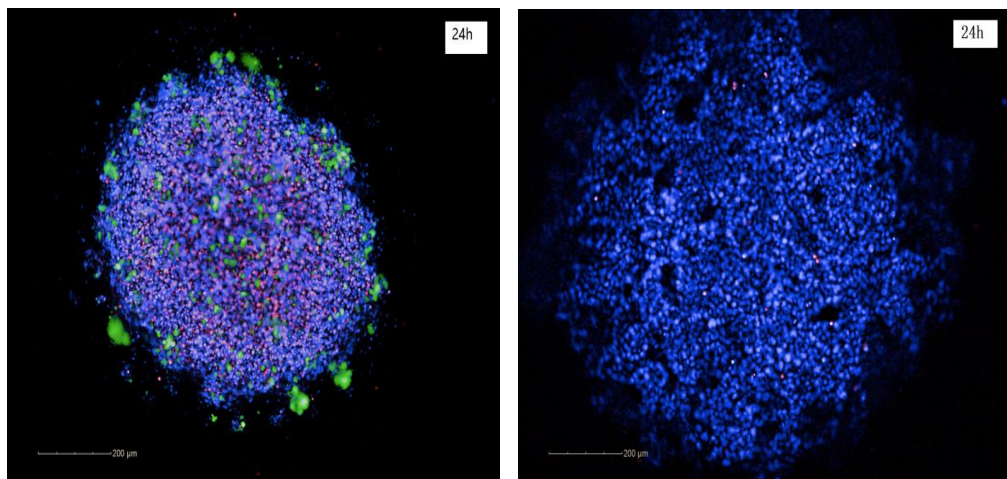
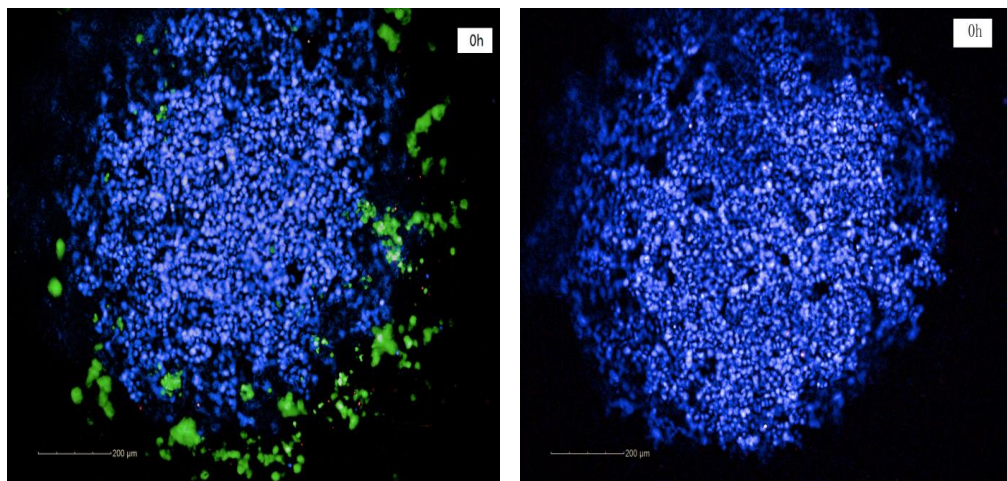
Low concentration of Oleic Acid

High concentration of Oleic Acid

3D原代肝微组织细胞毒性研究

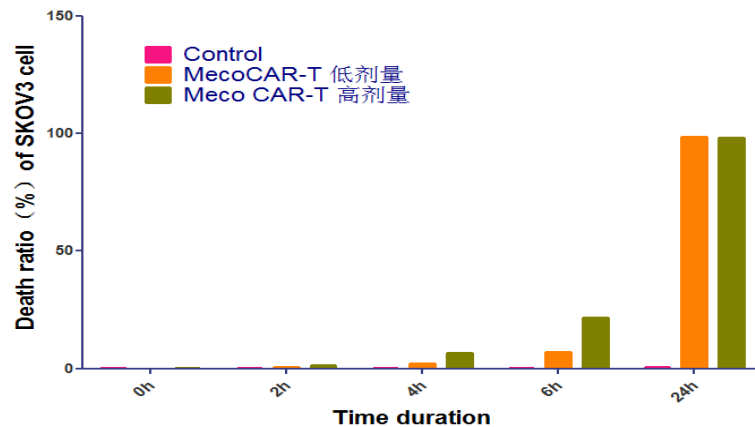


HCS 3D 肿瘤细胞凋亡 & Car-T细胞浸润分析

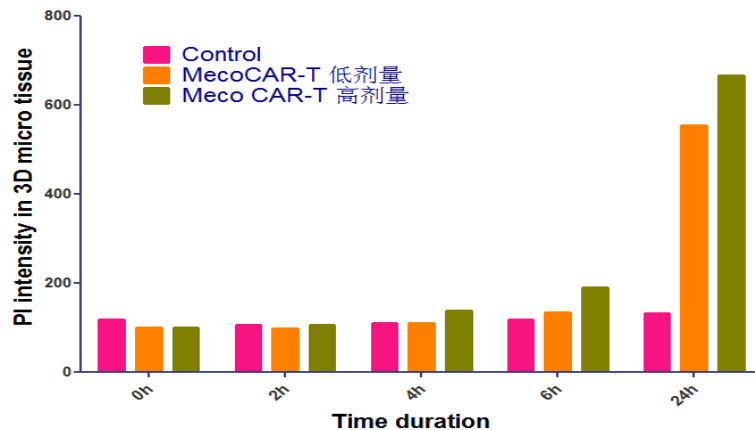


蓝色 : Hochest (肿瘤细胞) 绿色 : CAR T细胞 红色 : PI (死细胞)

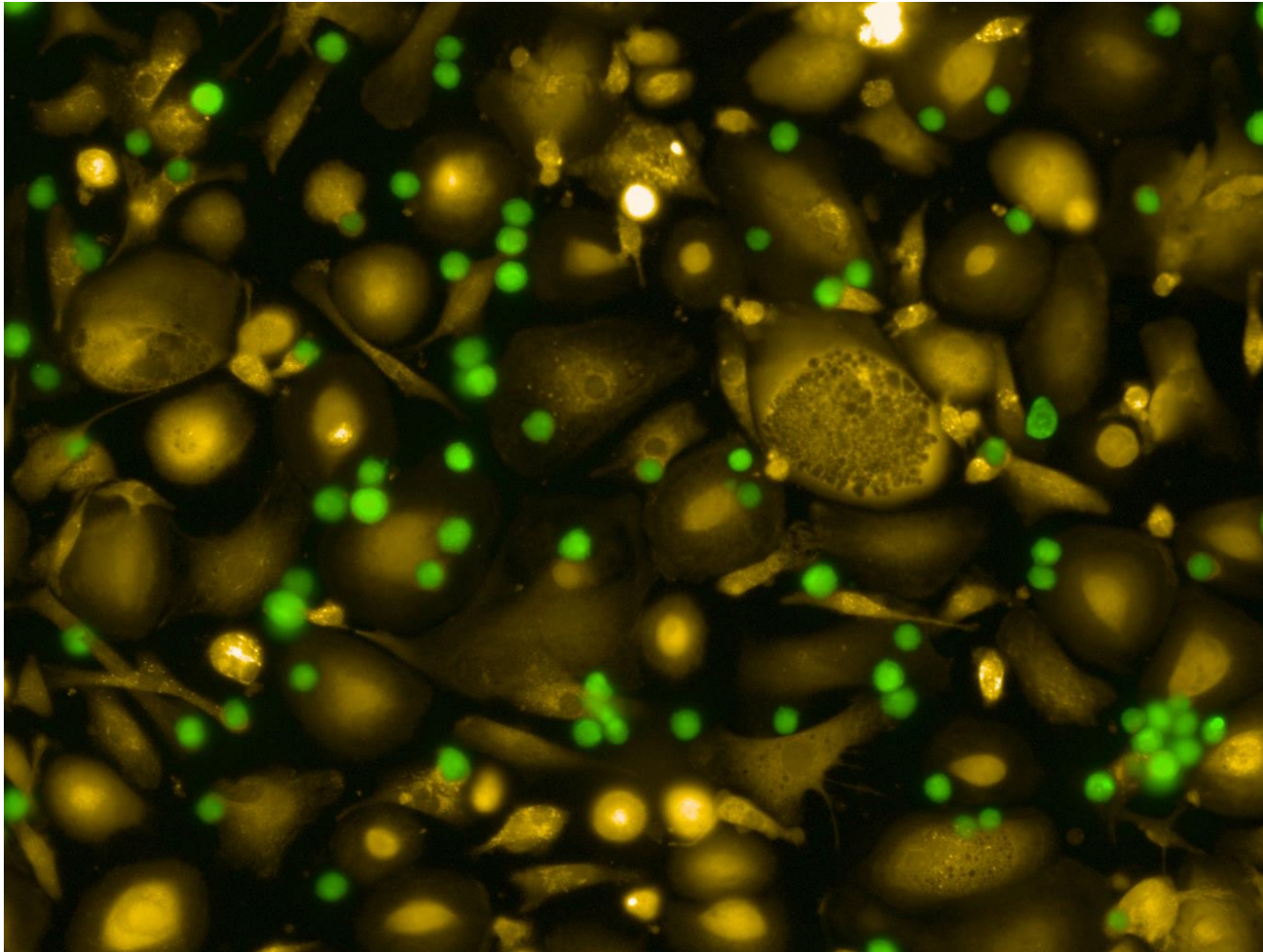
MecoCAR-T对 SKOV3 3D 肿瘤细胞的杀伤
死细胞数统计



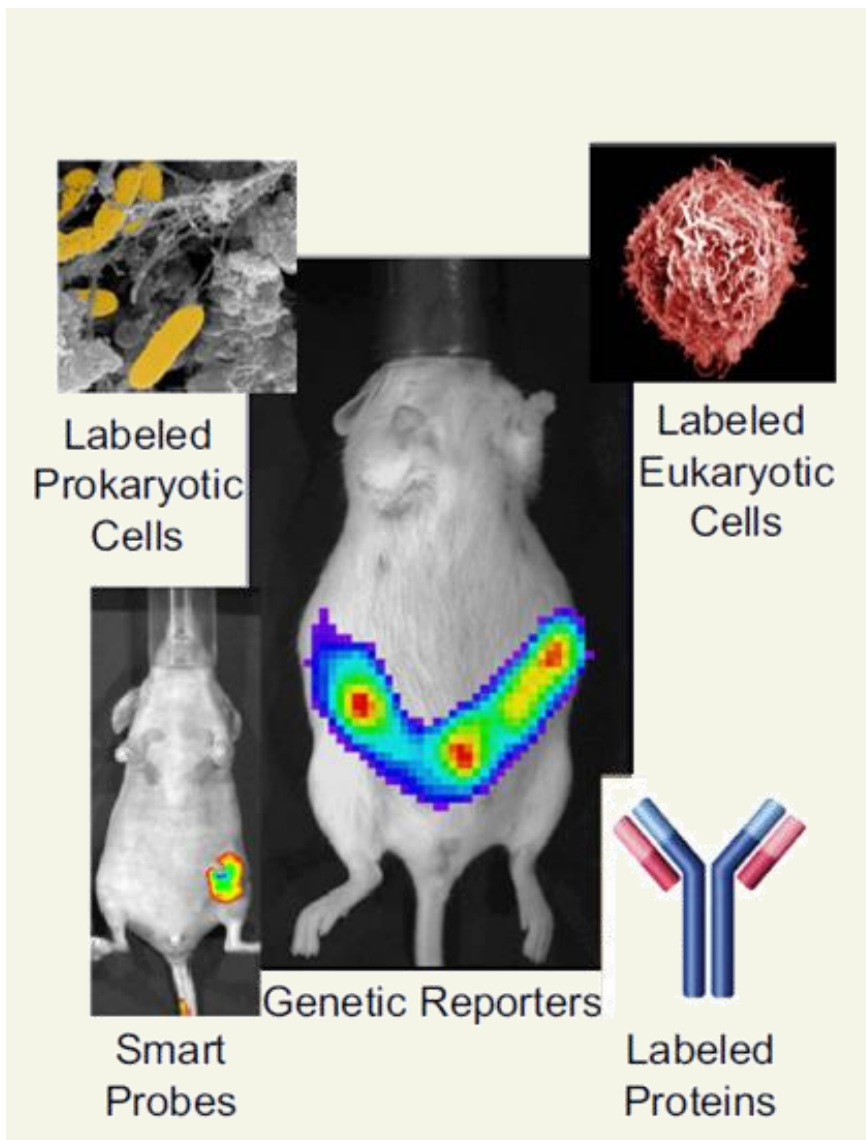
MecoCAR-T对 SKOV3 3D 肿瘤细胞的杀伤
PI荧光强度图



ADCP (Roche)



In Vivo Imaging Solution



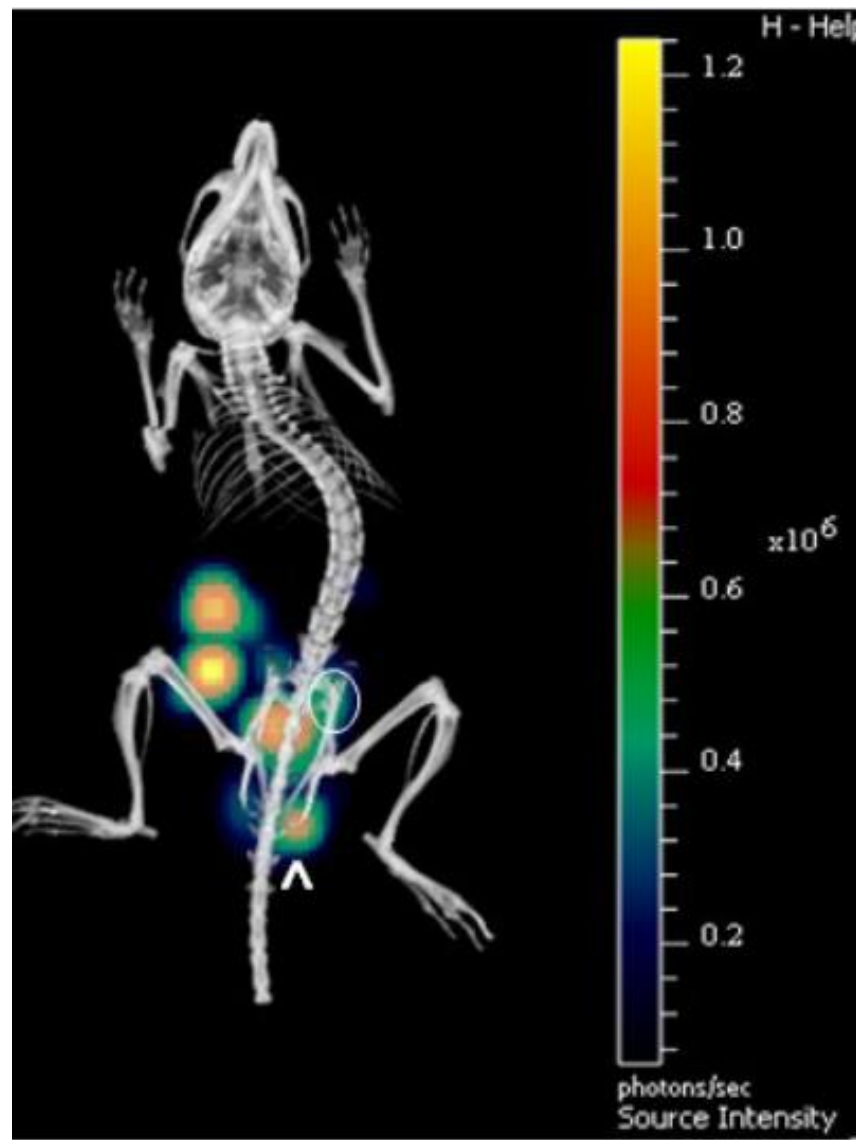
Labeled Prokaryotic Cells

Labeled Eukaryotic Cells

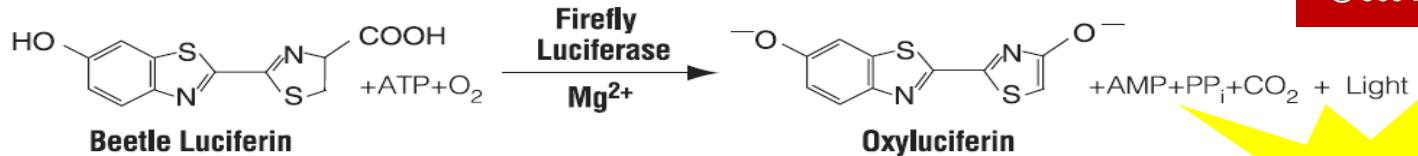
Smart Probes

Genetic Reporters

Labeled Proteins

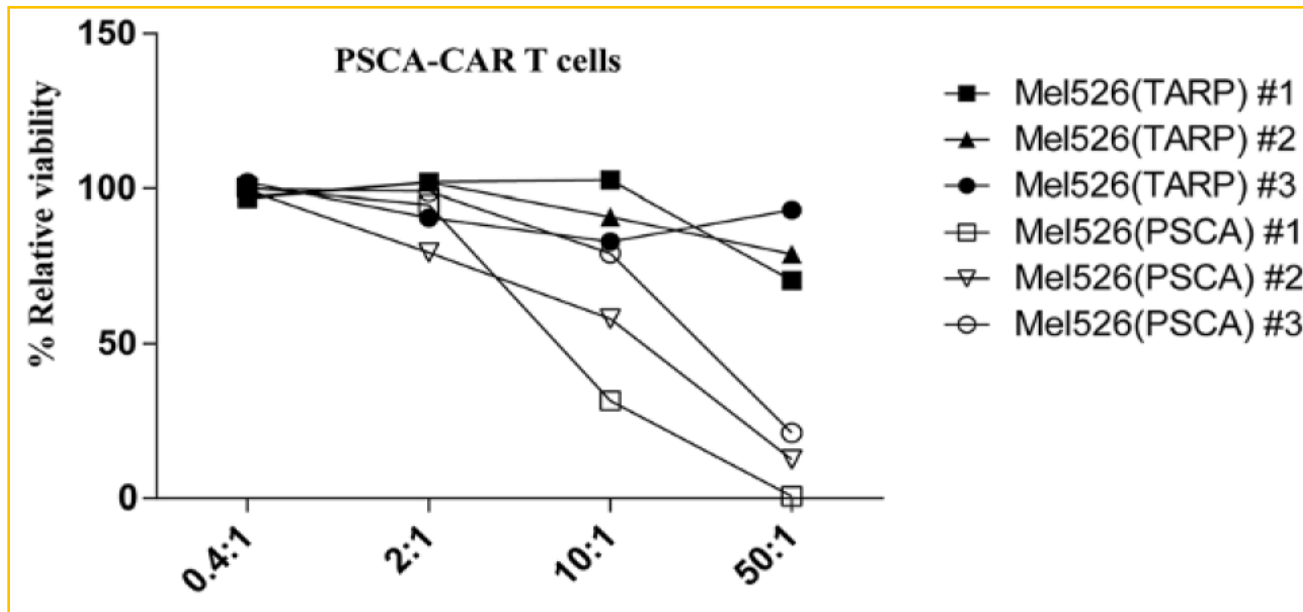


Luciferase-based Killing Assays

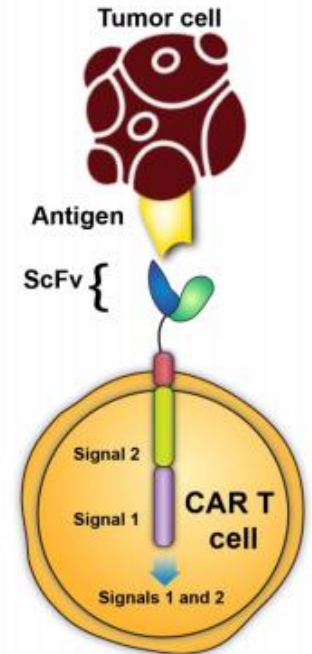


@ 560 nm

Luciferase labeled cell



PSCA-CAR T cells from three donors (#1, #2, #3) were co-cultured with luciferase-expressing mel526(PSCA) or mel526 (TARP) target cells for 2 days.





Luciferase-based *in vitro* – *in vivo* Solution

Bioluminescence Detection



britelite plus

neolite

steadylite plus

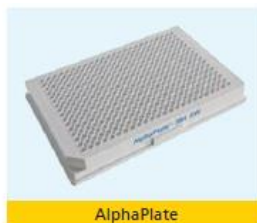
应用	报告基因检测		
荧光素酶	萤火虫荧光素酶		
信号半衰期	30 分钟	2.5 小时	5 小时
灵敏度	超高灵敏度	高灵敏度	中等
步骤	1 步		
微孔板规格	96, 384, 1536		
PerkinElmer 发射光检测设备	Victor X EnVision EnSpire TopCount MicroBeta		



B&W IsoPlate



1/2 AreaPlate



AlphaPlate

Bioware for *in vitro* & *in vivo*

Product Number	Cell Line Product Description
BW119262	Bioware Brite MCF7-Red-FLuc
BW119266	Bioware Brite A549-Red-FLuc
BW119267	Bioware Brite LL/2-Red-FLuc
BW119276	Bioware Brite SKOV3-Red-FLuc
BW124087	Bioware Brite 4T1-Red-FLuc
BW124316	Bioware Brite NCI-H460-Red-FLuc
BW124317	Bioware Brite Colo205-Red-FLuc
BW124318	Bioware Brite HCT-116-Red-FLuc
BW124353	Bioware Brite HT-29-Red-FLuc
BW124577	Bioware Brite U87MG-Red-FLuc
BW124734	Bioware Brite B16F10-Red-FLuc
BW124735	Bioware Brite K562-Red-FLuc
BW125055	Bioware Brite LNCaP-Red-FLuc
BW125058	Bioware Brite BxPC3-Red-FLuc
BW128090	Bioware Brite 4T1-Red-FLuc-GFP
BW128092	Bioware Brite HT1080-Red-FLuc
BW128444	Bioware Brite PC3-Red-FLuc
BW133416	Bioware Brite PC3-Red-FLuc-GFP
BW134246	Bioware Brite GL261-Red-FLuc
BW134280	Bioware Brite HepG2-Red-FLuc
122799	XenoLight D-Luciferin (K+Salt)

Quantitative Pathological System

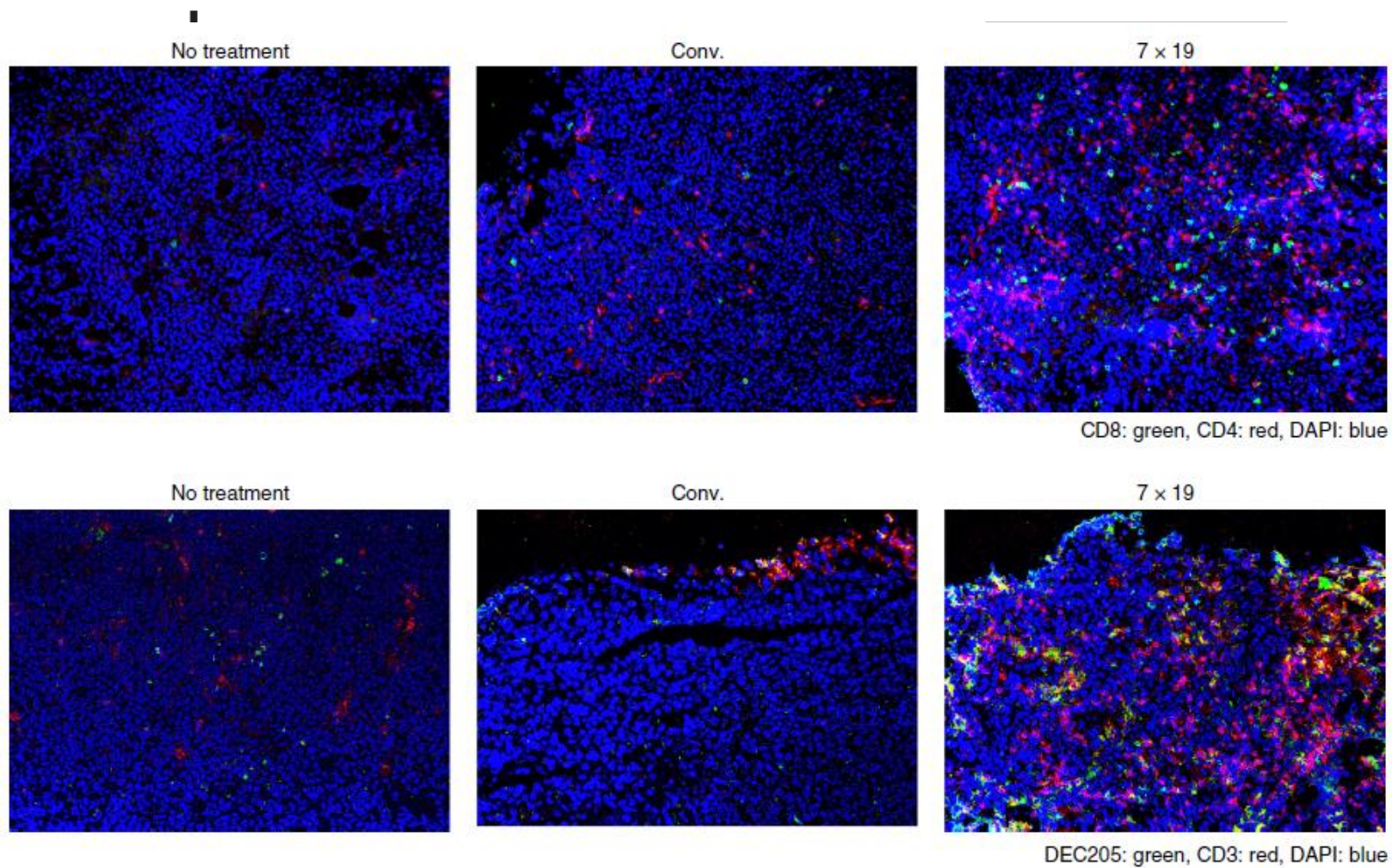
7

Breast Cancer
CD4(mouse)-Green
CD8(mouse)-Red
CD20(mouse)-Orange
FoxP3-(rabbit)-Purple
PD-L1(rabbit)-Yellow
CK(mouse)-Cyan
Nuclei(DAPI)-Blue

PLEX

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PerkinElmer CAR-T细胞治疗研发支持方案

