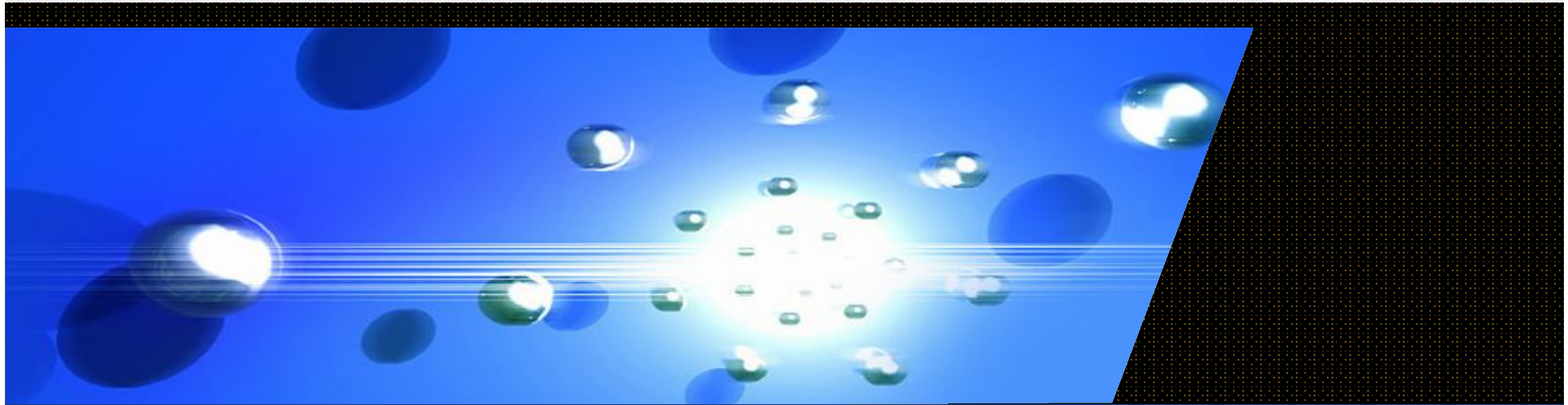
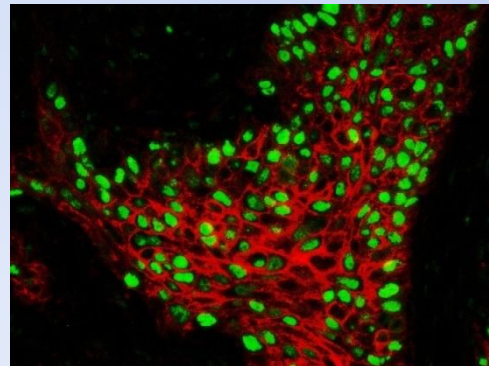
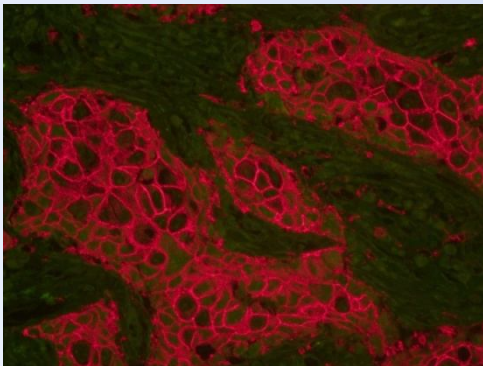




武汉珈源量子点公司 Jiayuan Quantum Dots



## 量子点免疫荧光试剂盒 (QDots-IF™)



生物医学事业部

<http://www.qds.net.cn>

Version 1, 2011.09

# 内容提要

珈源量子点介绍

试剂盒简介

- 量子点免疫荧光单染试剂盒
- 量子点免疫荧光双染试剂盒
- 量子点荧光原位杂交试剂盒

应用举例



# 量子点——理想的荧光标记试剂

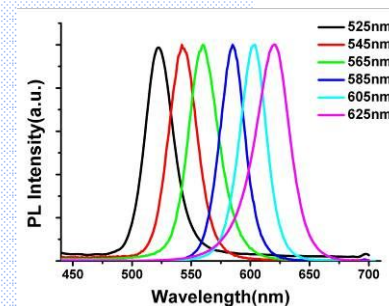
**量子点** (Quantum Dots) 又称半导体纳米晶，是一类由 II-VI 族和 III-V 族元素组成的纳米颗粒。生物标记中常见的 CdSe 量子点粒径在 1.8nm-7.0nm 变化时，其发射荧光可覆盖整个可见光区。

荧光发射波长可控、发射峰狭窄对称

激发谱宽而连续，消光系数大、荧光强度高

光稳定性好、易与生物分子偶联

多色标记成像、长时动态监测



# 量子点在生物医学上的应用

蛋白印迹

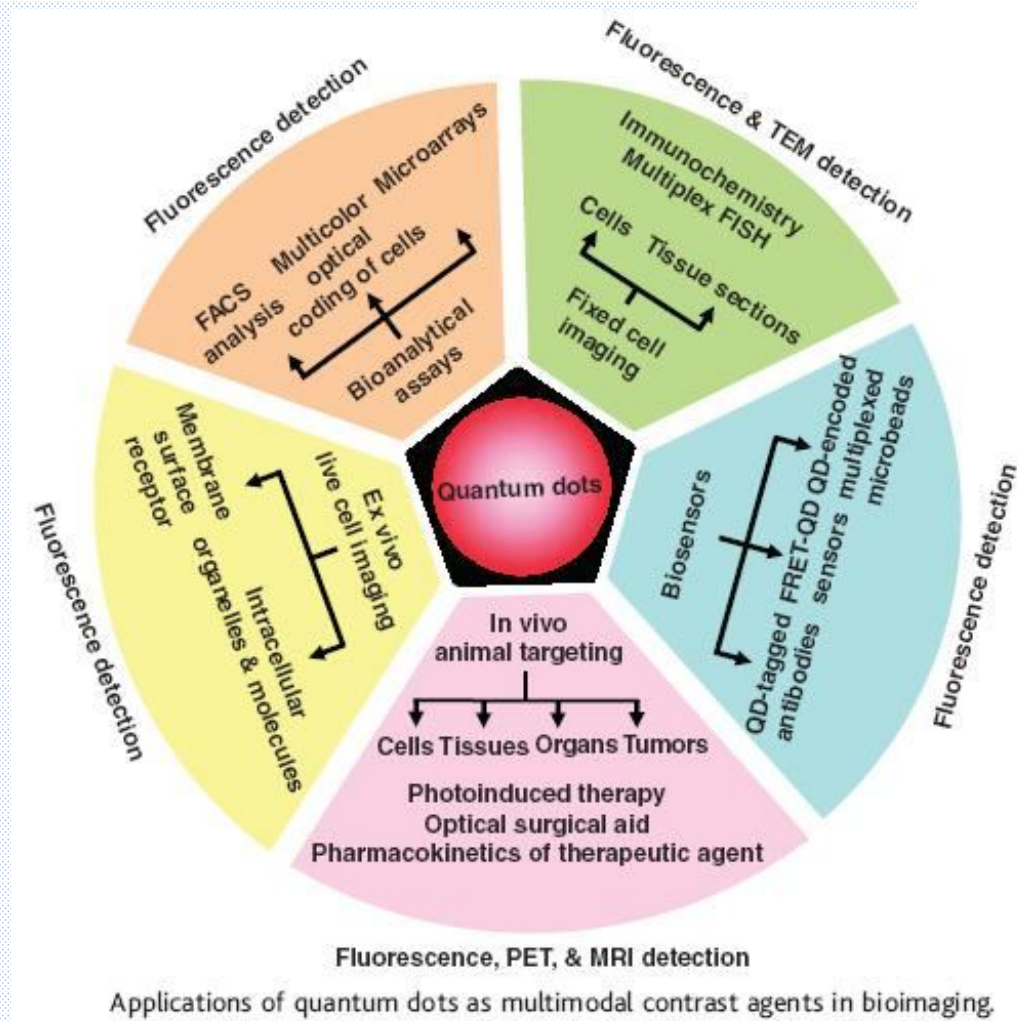
免疫荧光

活细胞示踪

活体成像

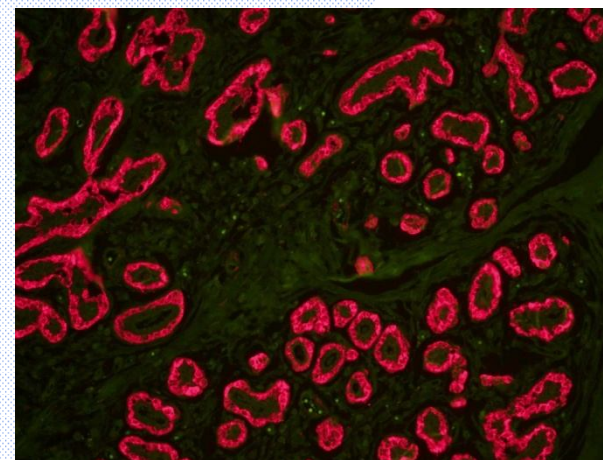
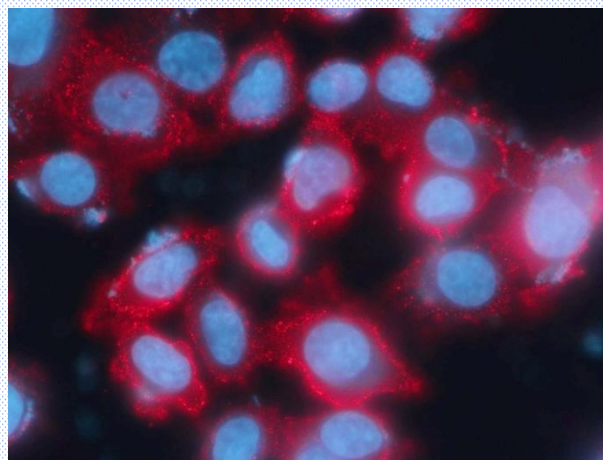
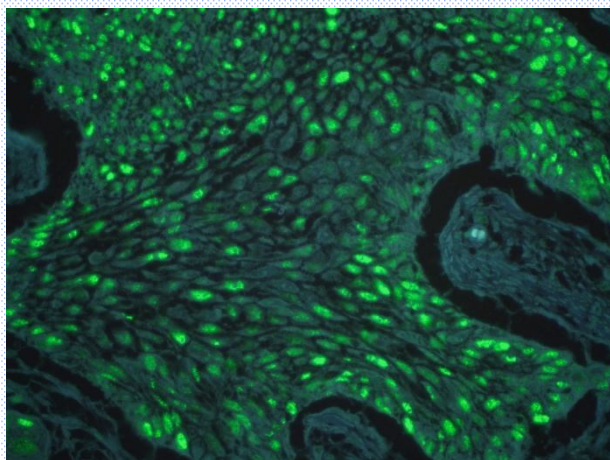
病原微生物检测

生物传感器



# 量子点免疫荧光单染系列试剂盒

产品编号	产品名称	荧光最大发射波长	激发波长
QK525M	量子点超敏荧光试剂盒 (QDs-G/M IgG) -525	525nm	≤485, 紫外
QK525R	量子点超敏荧光试剂盒 (QDs-G/R IgG) -525	525nm	≤485, 紫外
QK605M	量子点超敏荧光试剂盒 (QDs-G/M IgG) -605	605nm	≤565, 蓝光
QK605R	量子点超敏荧光试剂盒 (QDs-G/R IgG) -605	605nm	≤565, 蓝光
QK525S	量子点超敏荧光试剂盒 (QDs-SA) -525	525nm	≤485, 紫外
QK605S	量子点超敏荧光试剂盒 (QDs-SA) -605	605nm	≤565, 蓝光



# 量子点单染试剂盒组成成分

## ◆ Cat. QK525M QK605M QK525R QK605R

试剂A Tween 20

试剂B 缓冲液：2% BSA（用于封闭和稀释试剂）

试剂C **QDs-IgG复合物（1 $\mu$ M）**

试剂D 通透液：0.1% Triton-X 100

试剂E 缓冲甘油封固剂

## ◆ Cat. QK525S QK605S

试剂A Tween 20

试剂B 缓冲液：2% BSA（用于封闭和稀释试剂）

试剂C 生物素化二抗（进口分装）

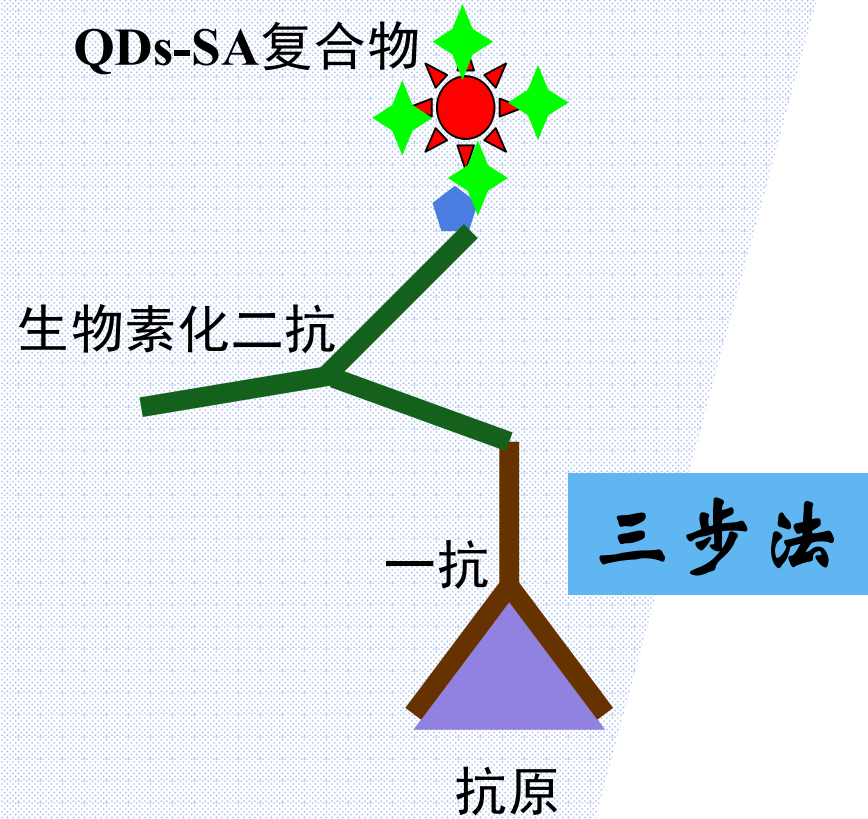
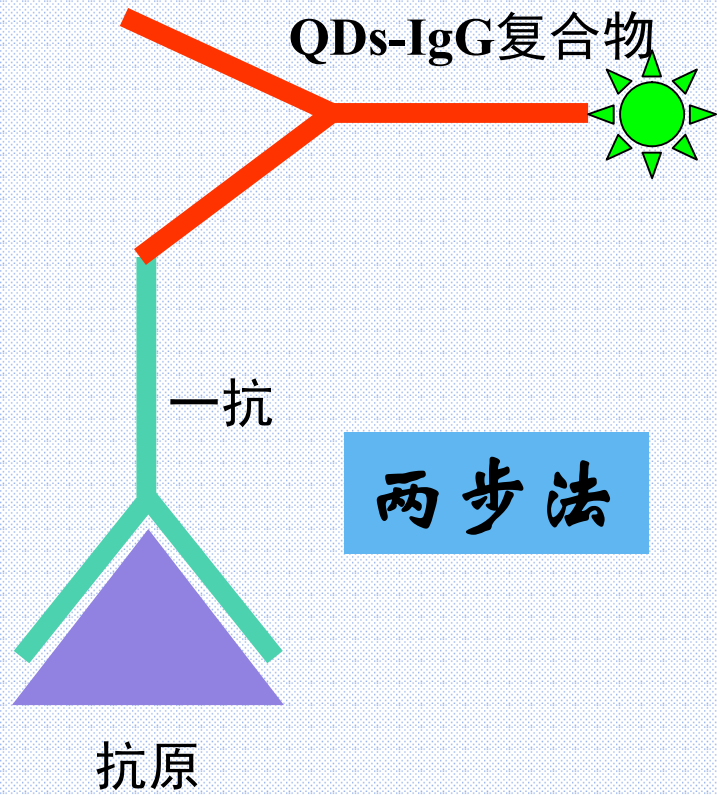
试剂D **QDs-SA复合物（1 $\mu$ M）**

试剂E 通透液：0.1% Triton-X 100

试剂F 缓冲甘油封固剂



# 原理及适用范围



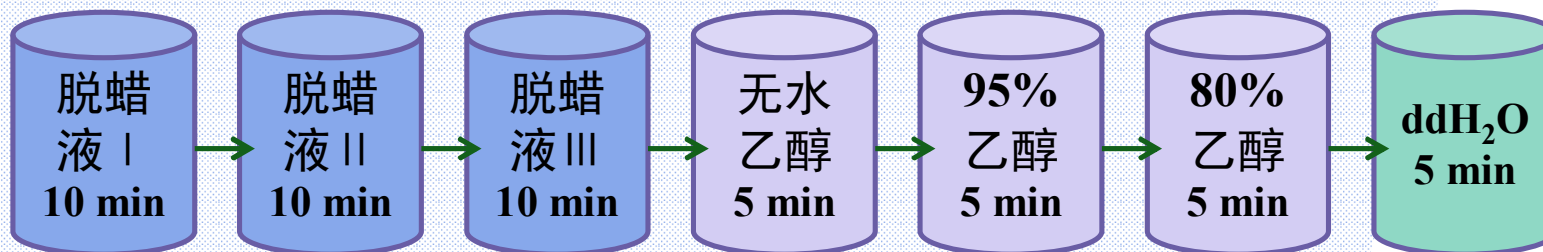
适用范围：冰冻切片、石蜡包埋组织切片、固定细胞、活细胞等



# 操作流程

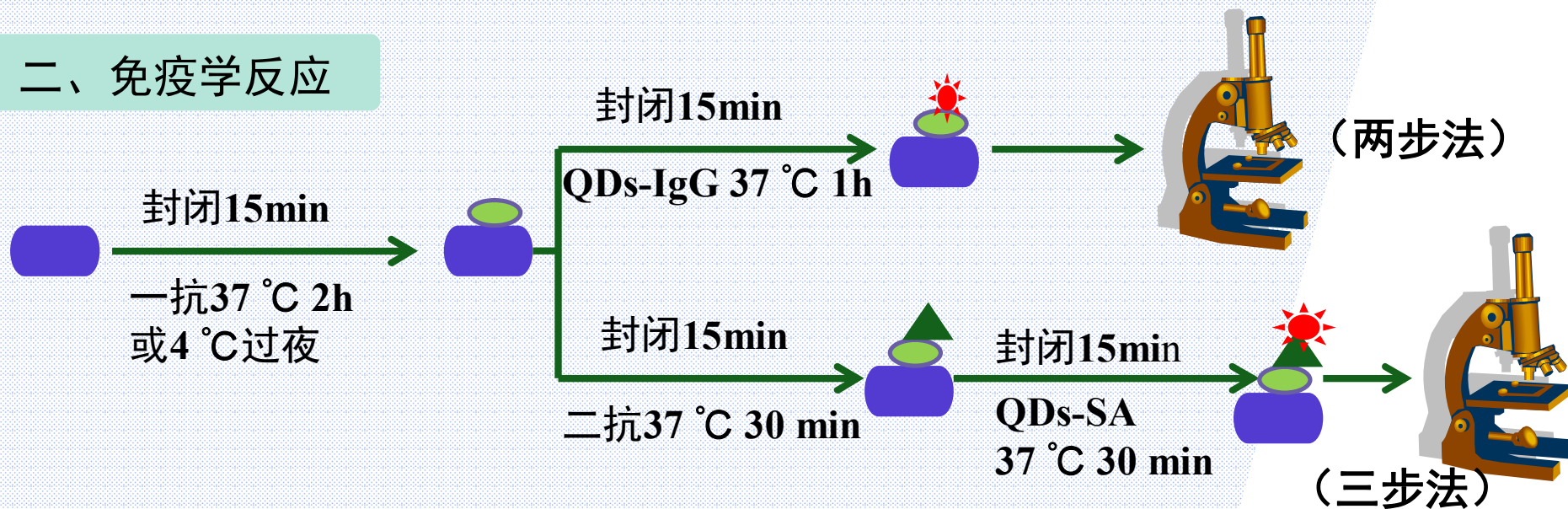
## 一、组织预处理

### 1. 脱蜡入水



### 2. 抗原修复：微波或高压

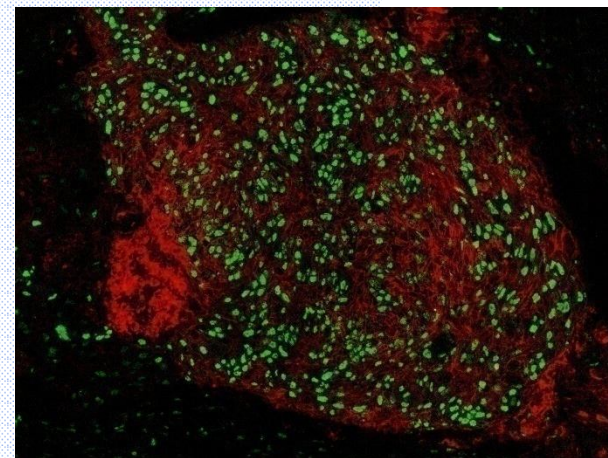
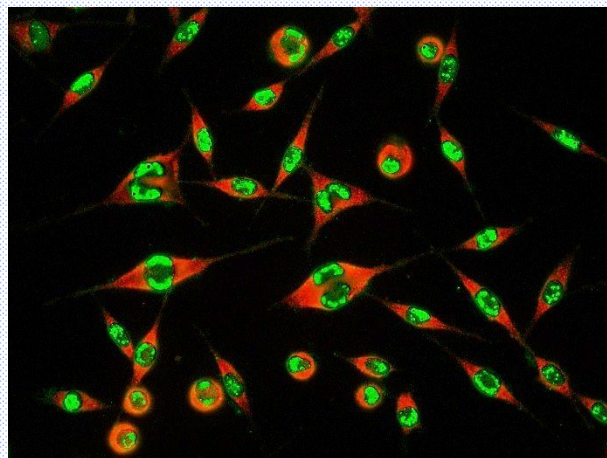
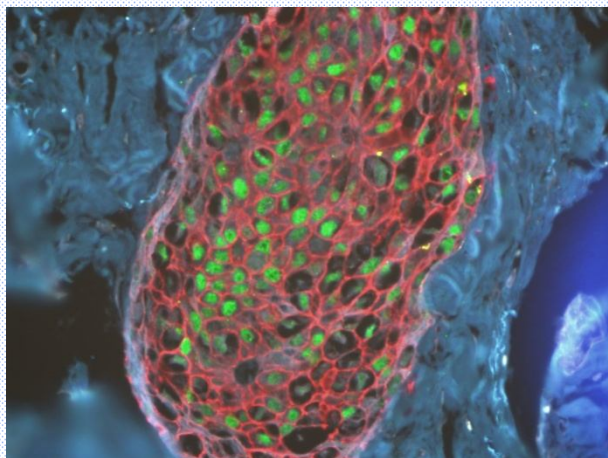
## 二、免疫学反应





# 量子点免疫荧光双染系列试剂盒

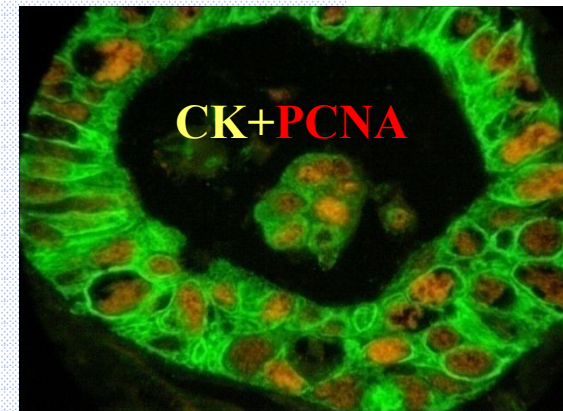
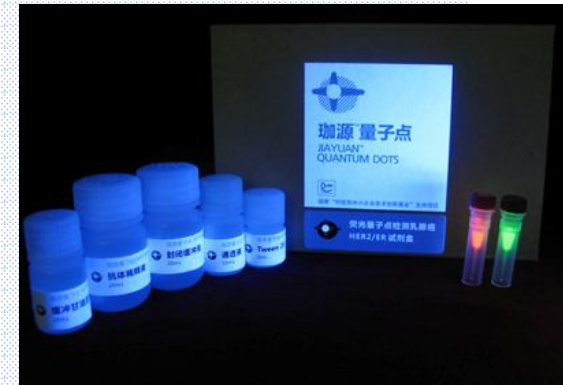
货号	名称	荧光最大发射波长	激发波长
QK525MD	量子点双染荧光试剂盒 (605SA+ 525-G/M IgG)	—	≤485, 紫外
QK605MD	量子点双染荧光试剂盒 (525SA+ 605-G/M IgG)	—	≤485, 紫外
QK525RD	量子点双染荧光试剂盒 (605SA+ 525-G/R IgG)	—	≤485, 紫外
QK605RD	量子点双染荧光试剂盒 (525SA+ 605-G/R IgG)	—	≤485, 紫外



# 量子点双染试剂盒

## 组成成分

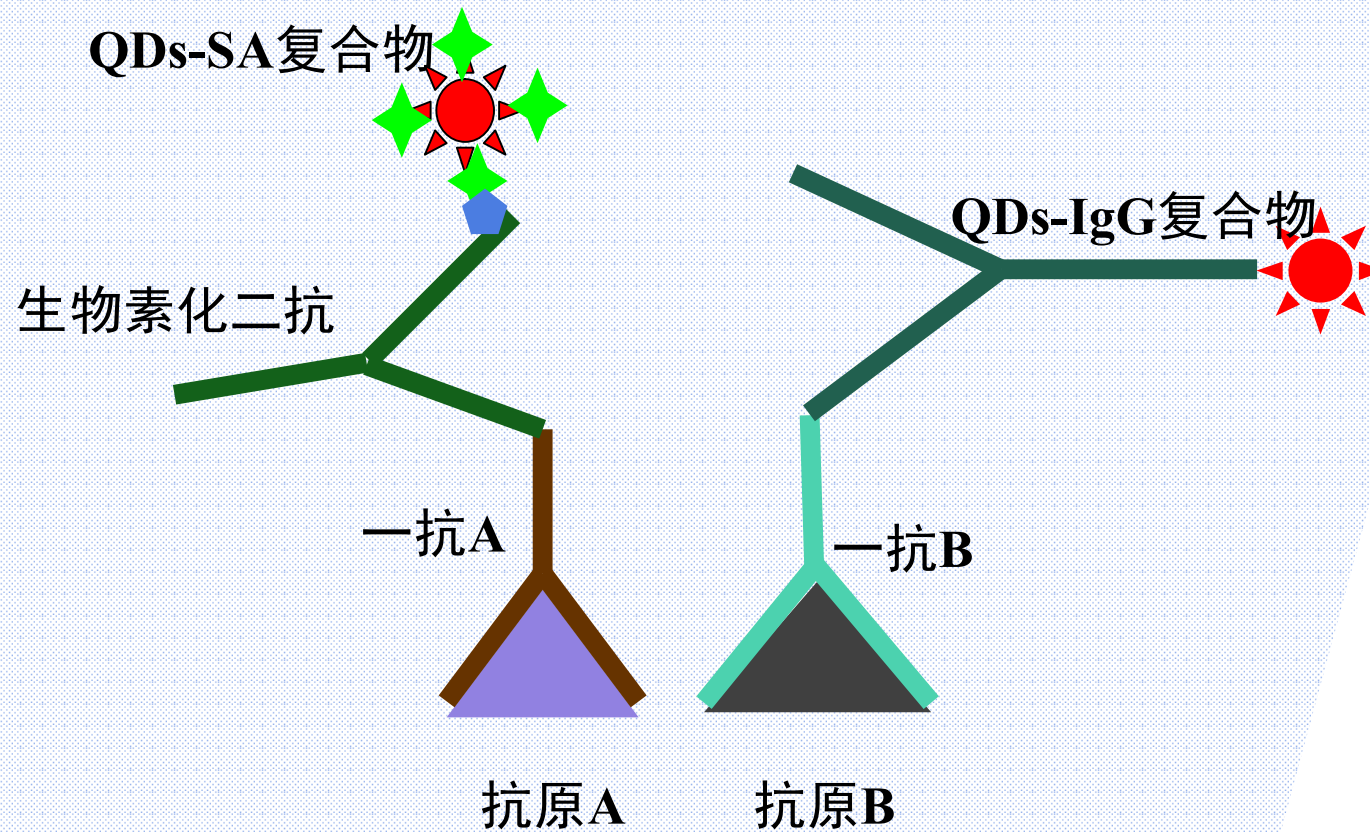
- 试剂A Tween 20
- 试剂B 缓冲液：2% BSA（用于封闭和稀释试剂）
- 试剂C 生物素化二抗（进口分装）
- 试剂D **QDs-SA复合物（1 $\mu$ M）**
- 试剂E **QDs-IgG复合物（1 $\mu$ M）**
- 试剂F 通透液：0.1% Triton-X 100
- 试剂G 缓冲甘油封固剂



★要求两种一抗来自不同种属



# 原理及适用范围



**一抗要求：**一抗源自不同种属

**适用范围：**冰冻切片、石蜡包埋组织切片、固定细胞、活细胞等

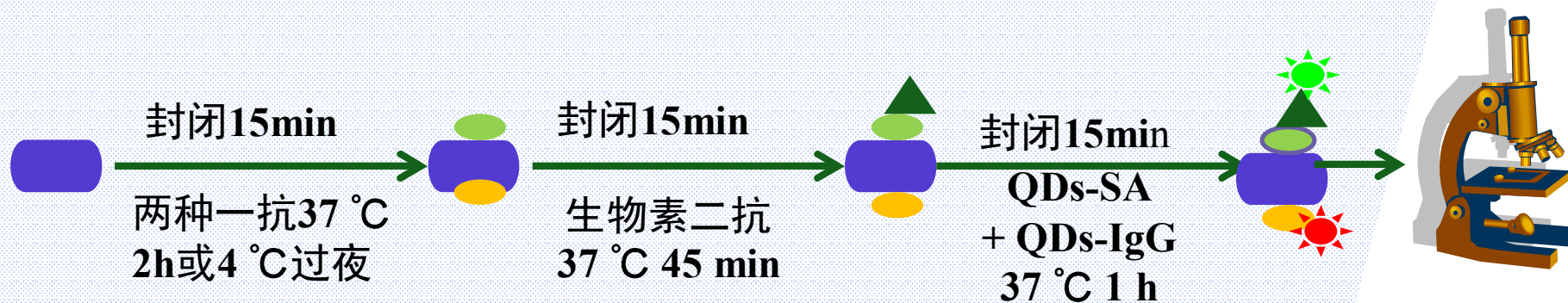


# 操作流程

## 一、组织预处理

同量子点免疫荧光单染

## 二、免疫学反应

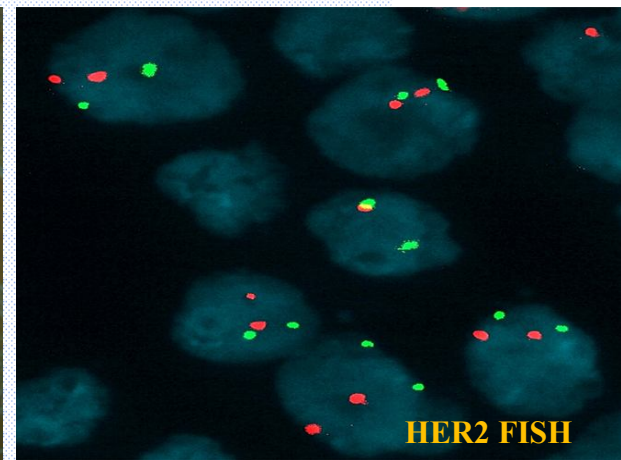
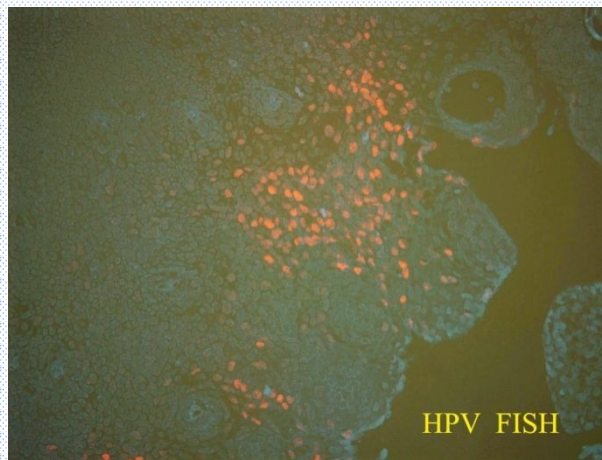
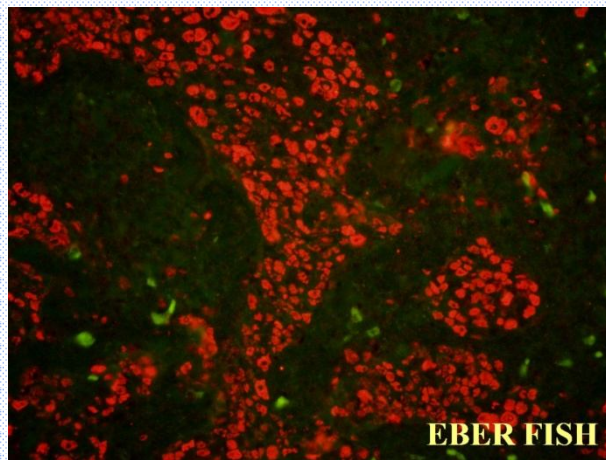


注：两种一抗同时孵育，例如抗体A和B均为浓缩型，稀释比例均为1:100，则在100  $\mu$ L稀释液中加入1  $\mu$ L抗体A和1  $\mu$ L抗体B，混匀后即可滴加于组织上。两种量子点配制方法同此。



# 量子点荧光原位杂交试剂盒

货号	名称	荧光最大发射波长	激发波长
QK525FB	量子点荧光原位杂交试剂盒 (QDs-FISH) -525	525	≤485, 紫外
QK605FB	量子点荧光原位杂交试剂盒 (QDs-FISH) -605	605	≤565, 紫外
QK525FD	量子点荧光原位杂交试剂盒 (QDs-FISH) -525	525	≤485, 紫外
QK605FD	量子点荧光原位杂交试剂盒 (QDs-FISH) -605	605	≤565, 紫外



# 量子点荧光原位杂交试剂盒组成成分

## ◆ Cat. QK525FB QK605FB

- 试剂A Tween 20
- 试剂B 缓冲液：2% BSA  
(用于封闭和稀释试剂)
- 试剂C 胃蛋白酶
- 试剂D 杂交后洗液
- 试剂E **QDs-IgG复合物 (1 μM)**
- 试剂F 缓冲甘油封固剂

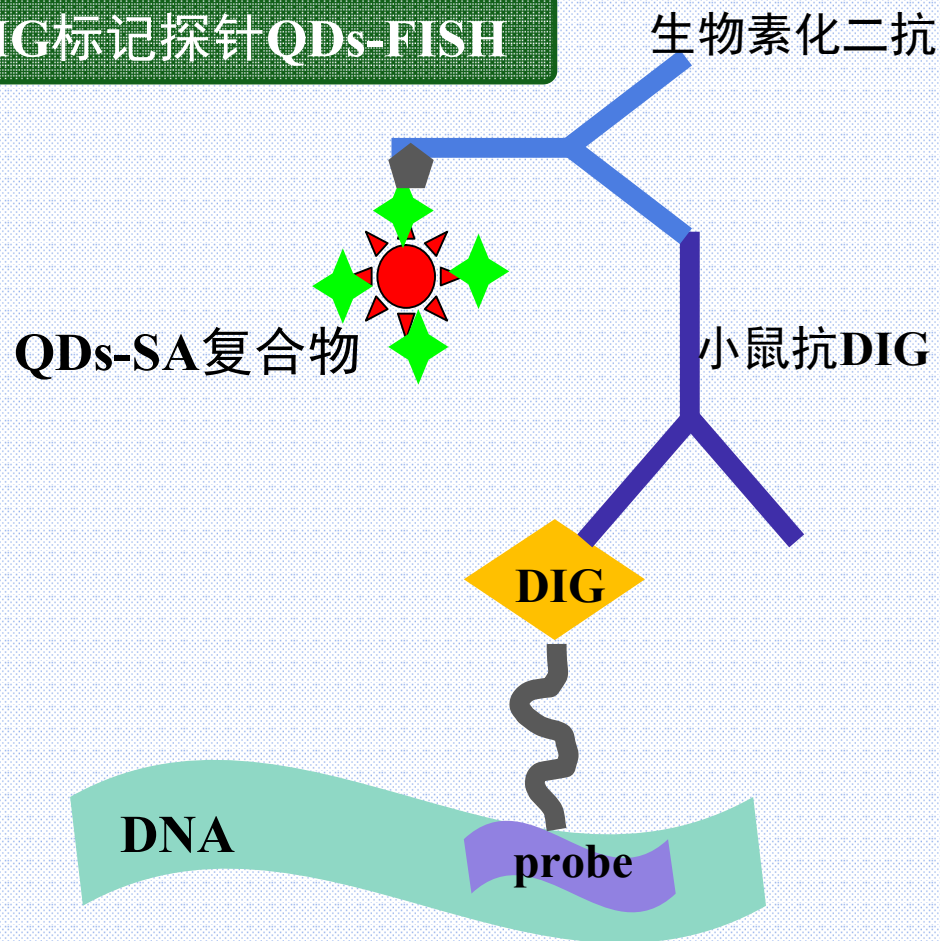
## ◆ Cat. QK525FD QK605FD

- 试剂A Tween 20
- 试剂B 缓冲液：2% BSA  
(用于封闭和稀释试剂)
- 试剂C 胃蛋白酶
- 试剂D 杂交后洗液
- 试剂E 小鼠抗DIG
- 试剂F 生物素化二抗 (进口分装)
- 试剂G **QDs-SA复合物 (1 μM)**
- 试剂H 缓冲甘油封固剂

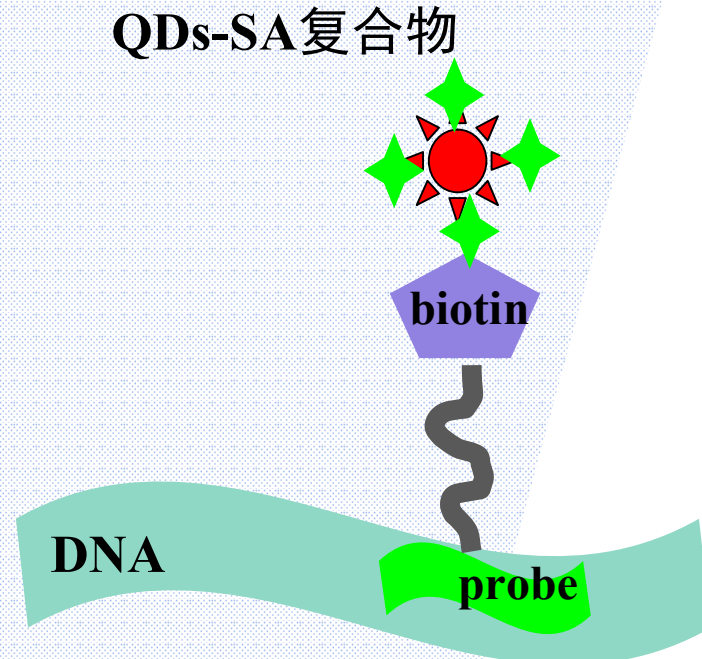


# 原理及适用范围

## DIG标记探针QDs-FISH



## biotin标记探针QDs-FISH



适用范围：冰冻切片、石蜡包埋组织切片、细胞等

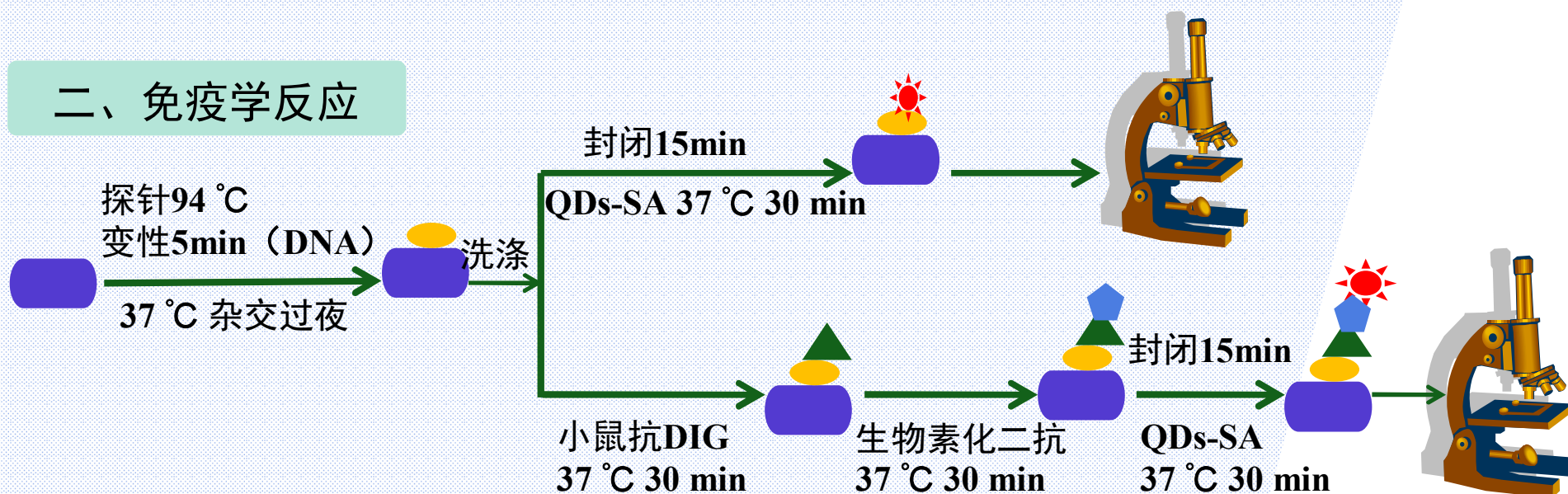


# 操作流程

## 一、组织预处理

脱蜡入水 → 热处理（微波） → 酶消化（37 °C 5~10 min） → 梯度酒精脱水

## 二、免疫学反应





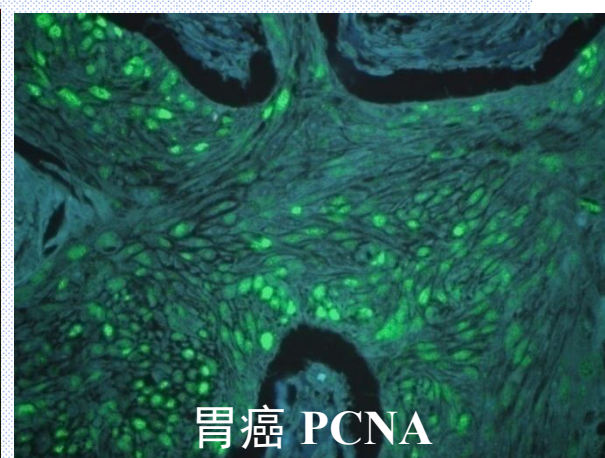
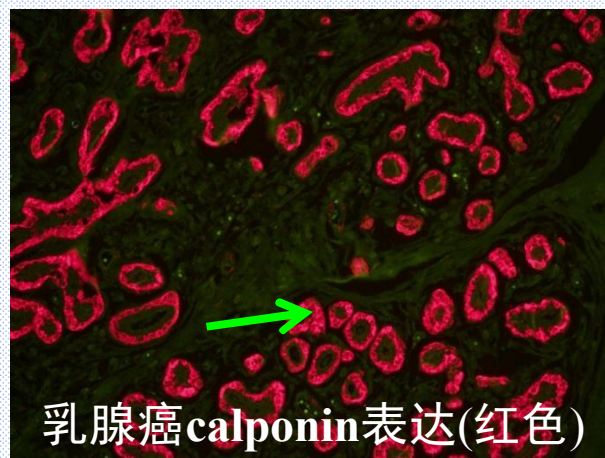
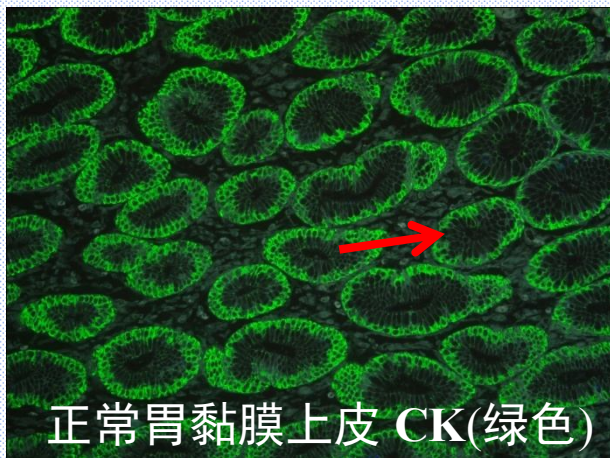
# 量子点作为免疫荧光染料的优势

- 适用范围广：石蜡包埋组织切片 新鲜组织 活细胞 固定细胞
- 灵敏度高，可检测低丰度抗原表达
- 特异性强，定位准确
- 信噪比高，可实现阳性信号与组织自发背景荧光完全区分
- 荧光稳定性好，无需避光操作--操作便利
- 可实现定量检测--量子点荧光定量免疫组织化学技术
- 可实现结果长期保存
- 可实现多种颜色一次同时成像
- 可实现抗原表达共定位分析（结合多光谱）

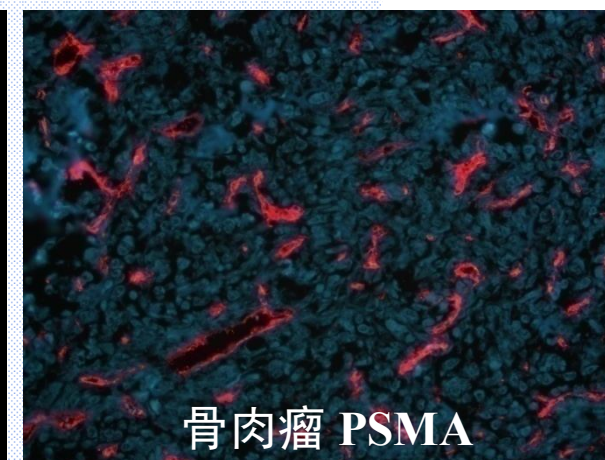
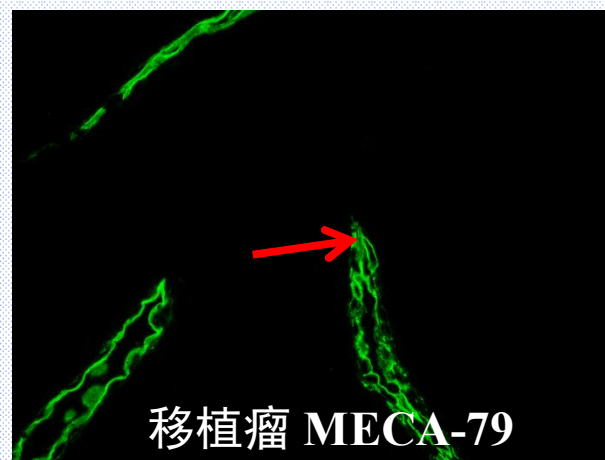
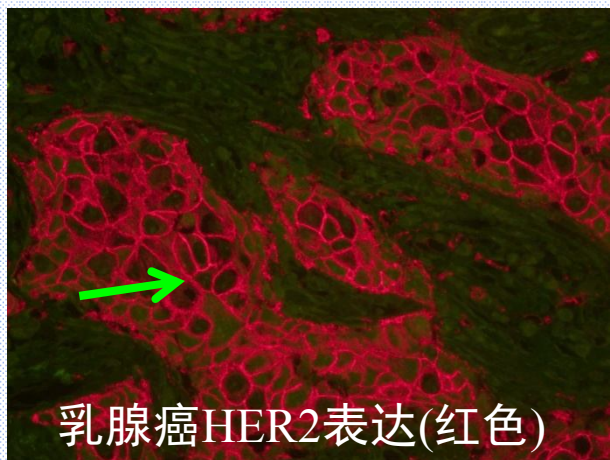


# 量子点免疫荧光适用范围广

## 石蜡包埋组织切片

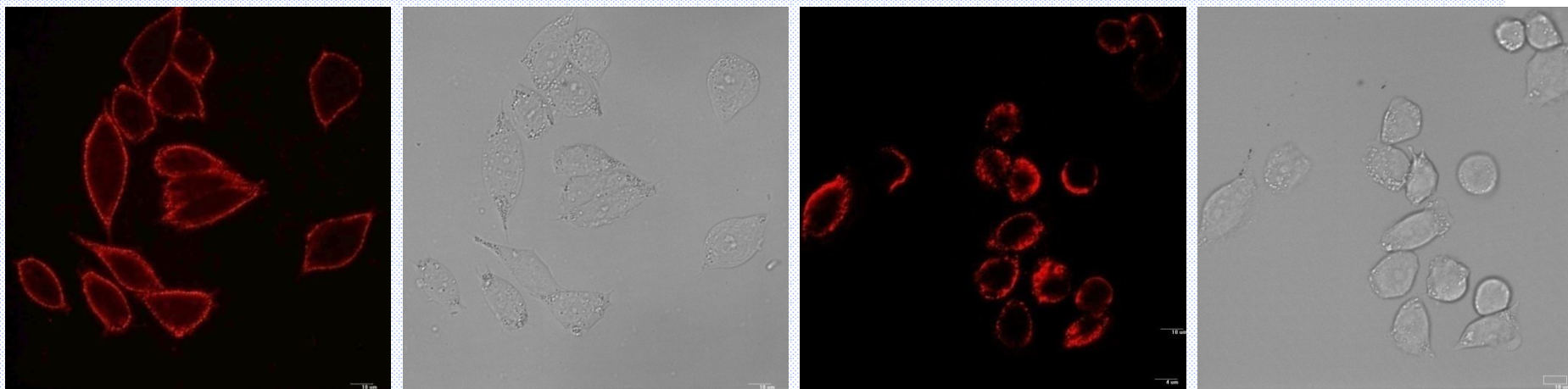


## 冰冻组织切片

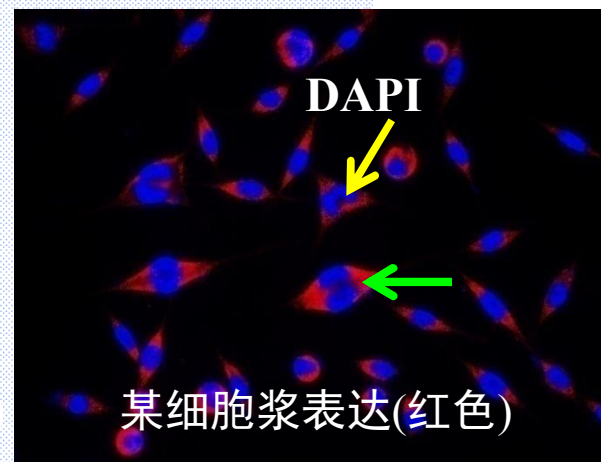
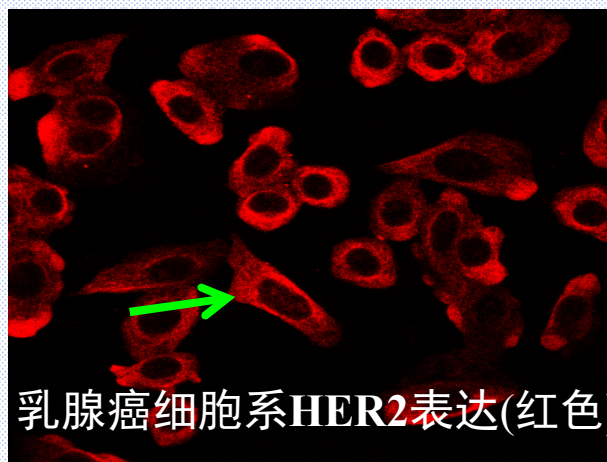
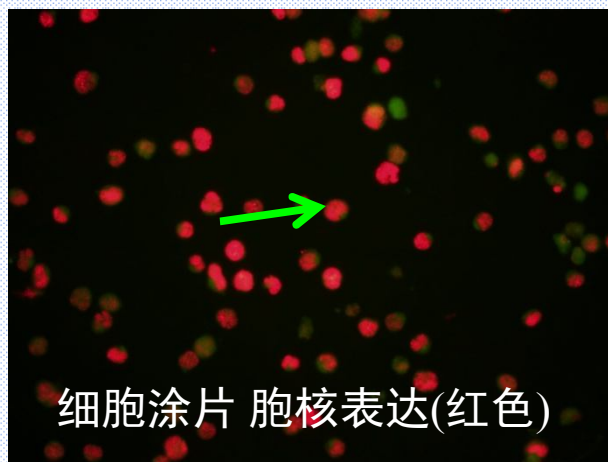


# 量子点免疫荧光适用范围广

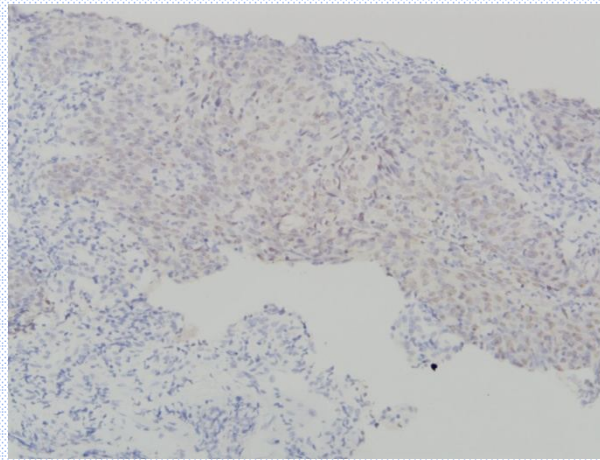
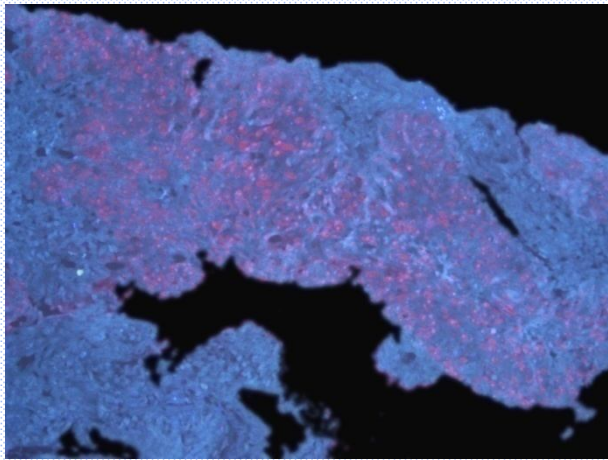
活细胞（黑色素瘤细胞CD146检测结果）



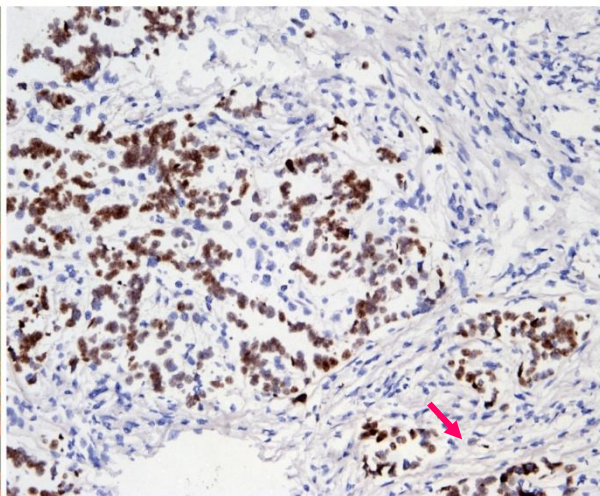
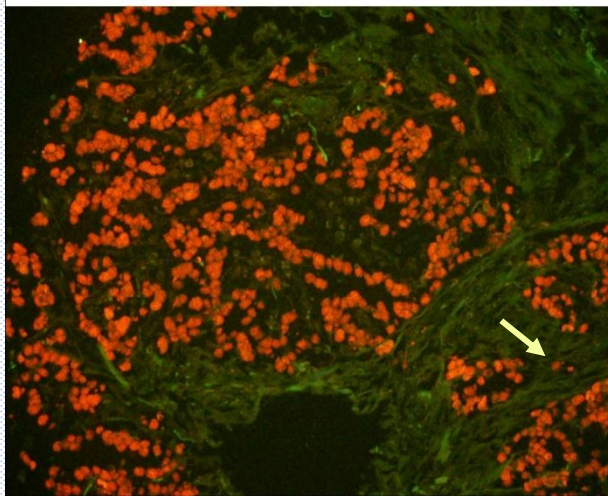
固定细胞



# 量子点免疫荧光灵敏度高



**Figure1. EBER FISH**在胃癌组织中的应用，并与CISH方法平行对比。



本公司产品与传统的方法相比具有相同的特异性，较高的灵敏性（**箭头所示**）。

*Experimental and Molecular Pathology*, 2010, 89, 367-371.

*J Nanosci Nanotechno*, 2011 .



# 量子点免疫荧光特异性强、定位准确

胞膜

胞浆

胞核

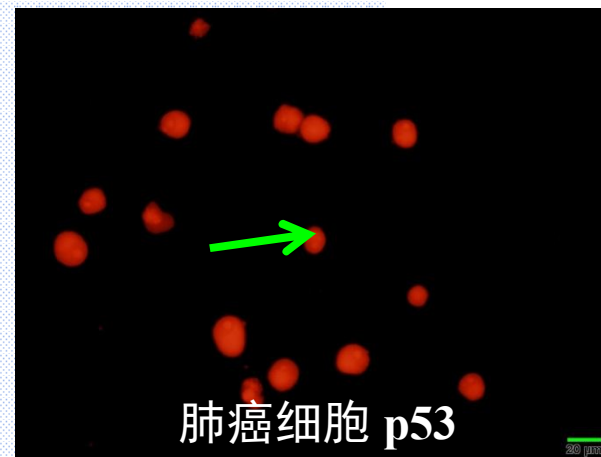
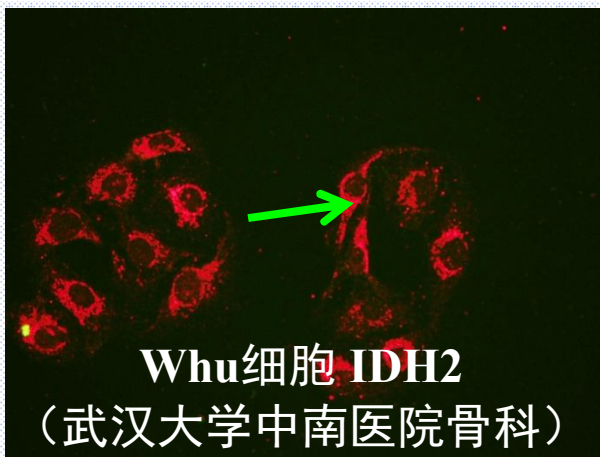
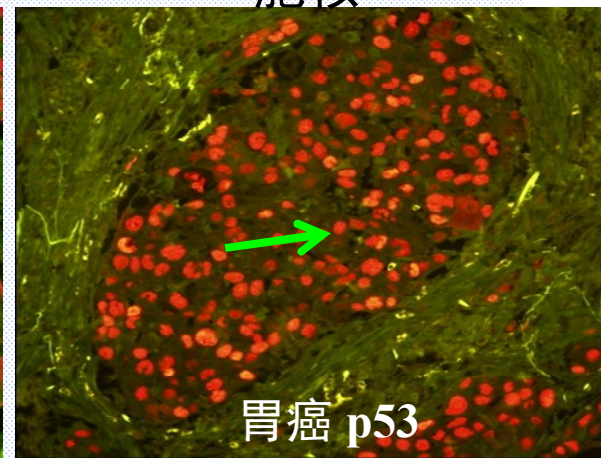
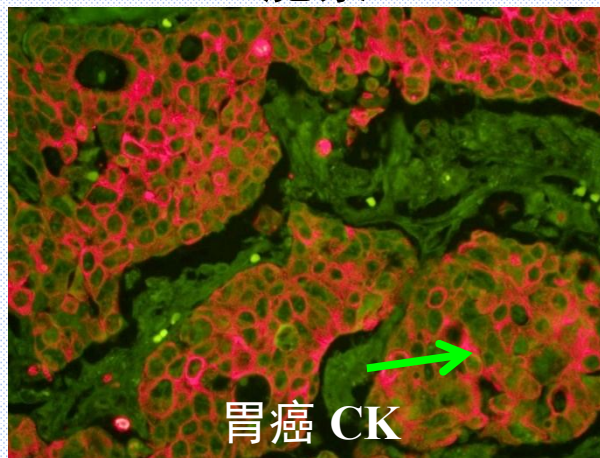
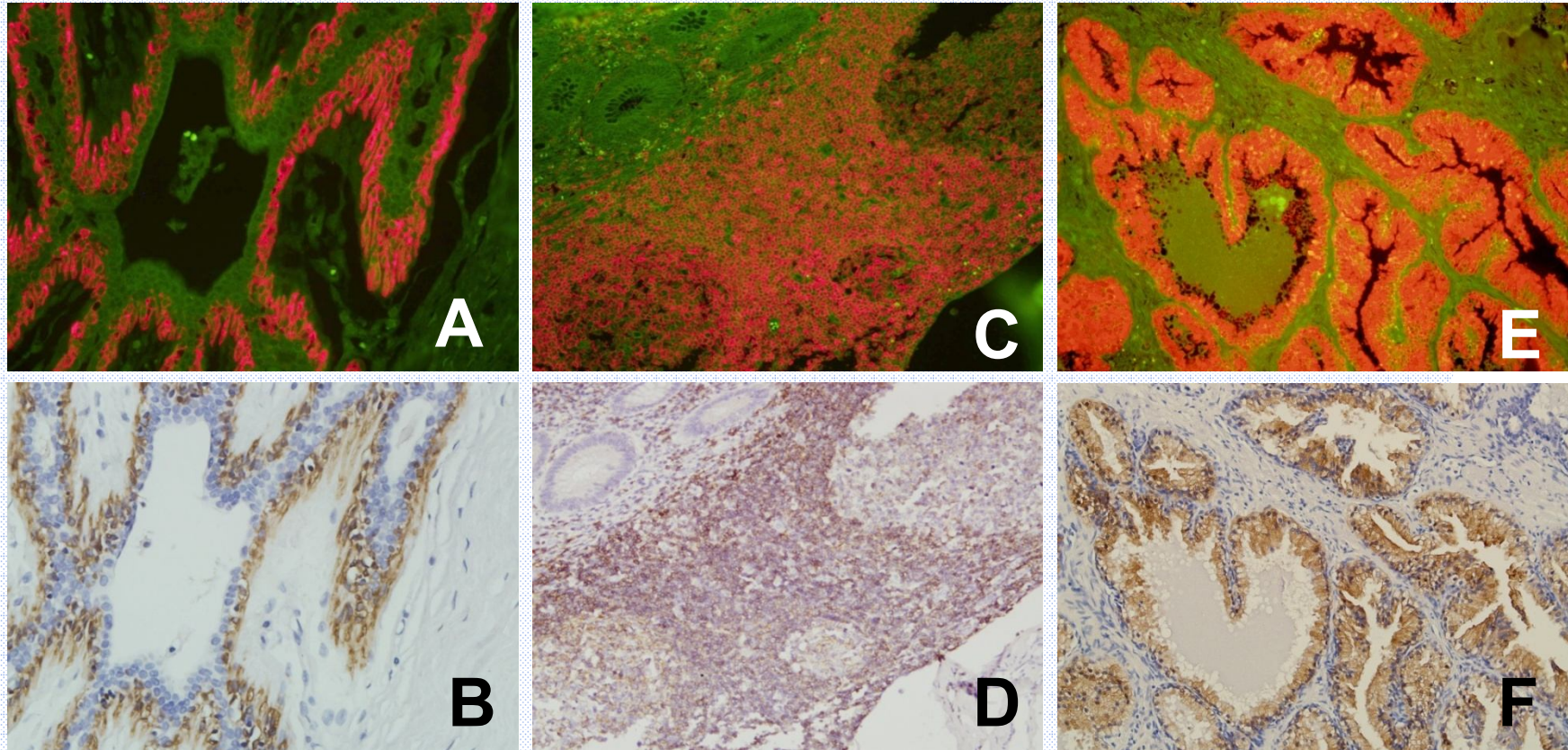


Figure 2. 量子点免疫荧光检测不同定位抗原的表达



# 量子点免疫荧光特异性强、定位准确

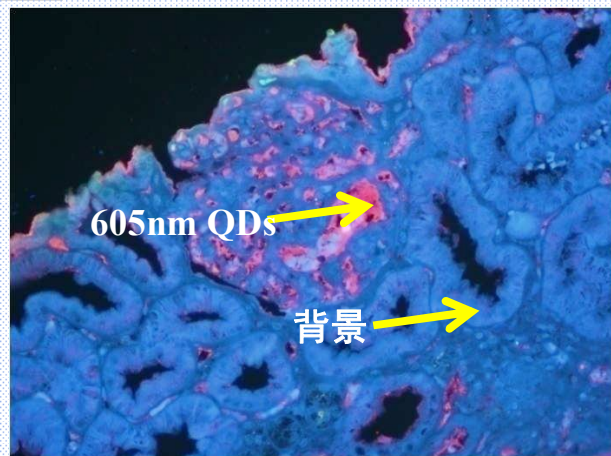


**Figure 3. 量子点免疫荧光与传统免疫组化对比**

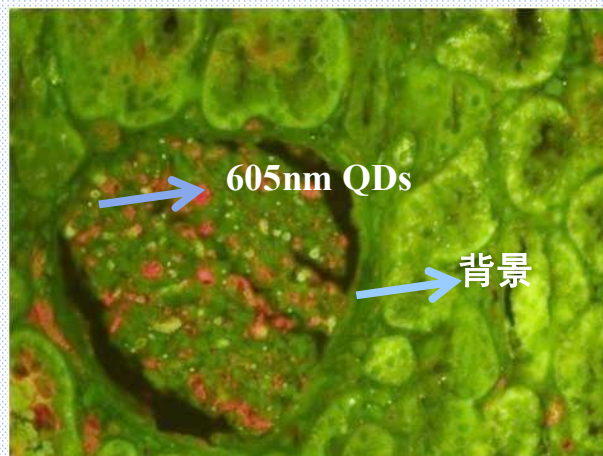
**AB: 乳腺癌 Calponin; CD: 阑尾 LCA; EF: 前列腺增生 PSA**  
**ACE: 量子点免疫荧光结果; BDF: 传统免疫组化结果**



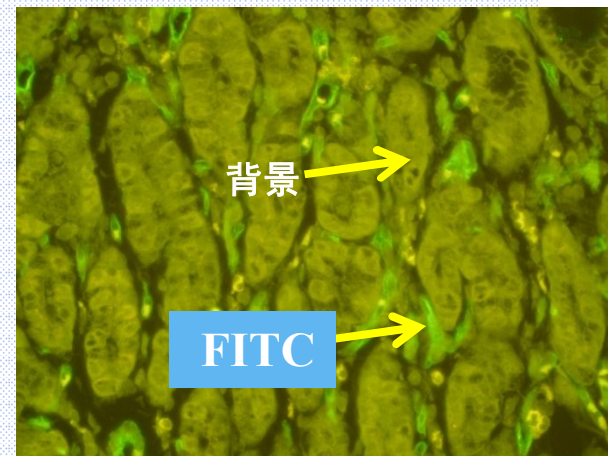
# 量子点免疫荧光信噪比高



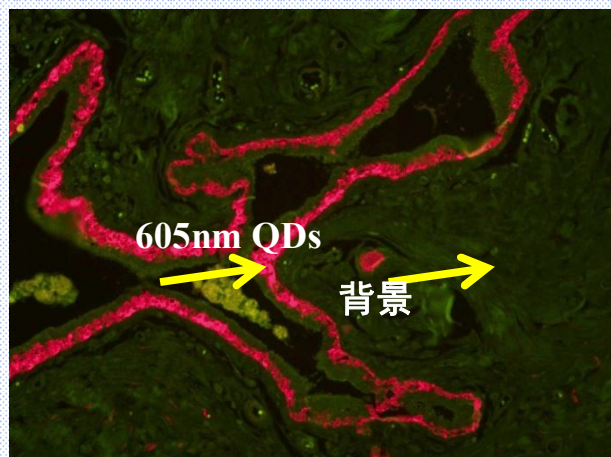
肾脏 IgA, 紫外光激发



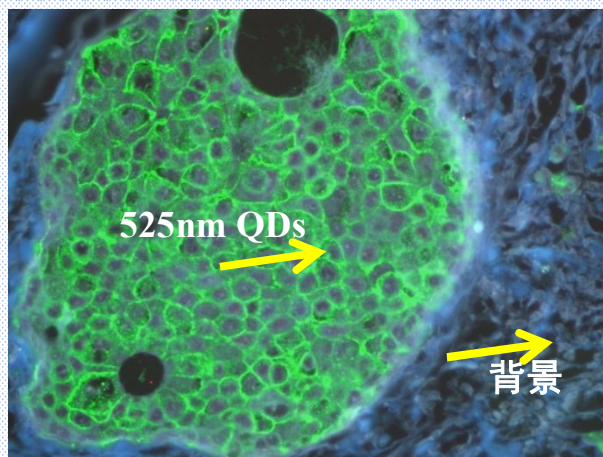
肾脏 IgA, 蓝光激发



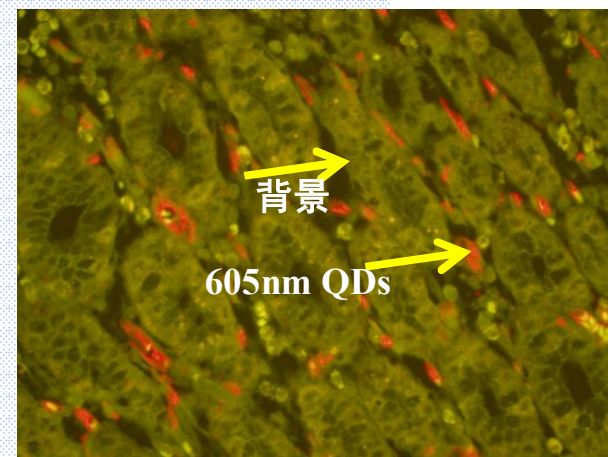
胃腺癌 caveolin1, 蓝光激发



正常乳腺 calponin, 紫外光激发  
红色: 605nm QDs; 绿色: 背景



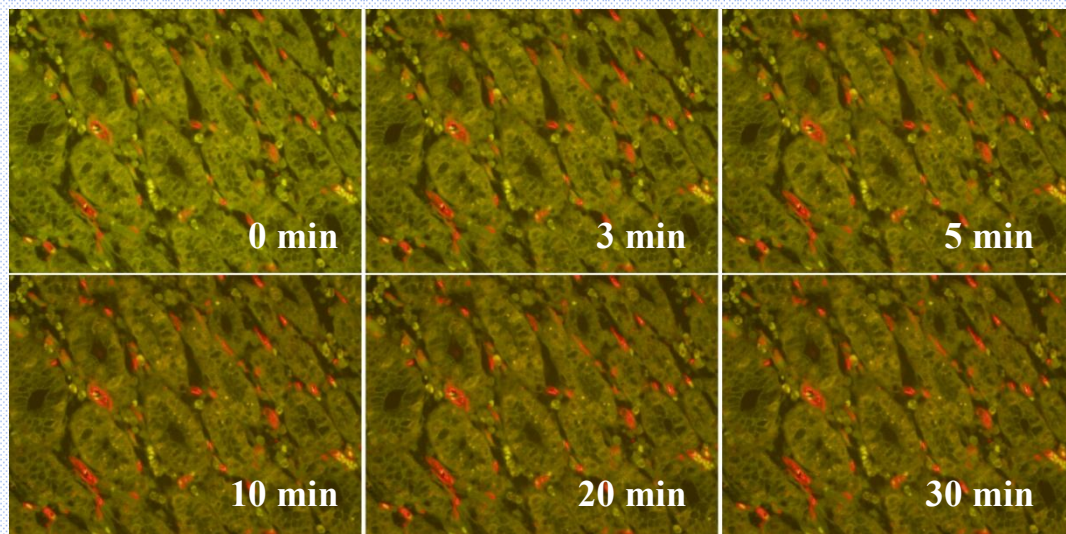
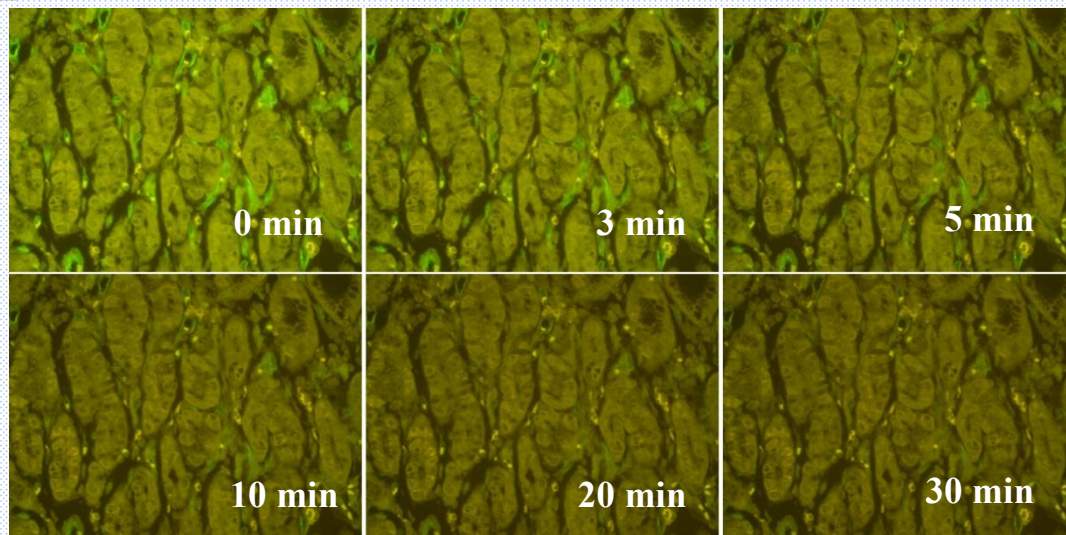
乳腺癌 HER2, 紫外光激发  
绿色: 525nm QDs; 蓝色: 背景



胃腺癌 caveolin1, 蓝光激发



# 量子点免疫荧光稳定性好



不同时间点  
FITC和QSA信号强度

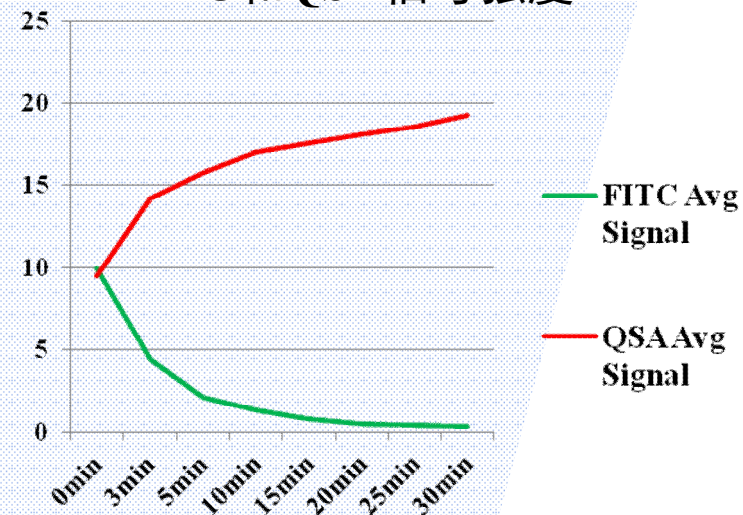


Figure 4. 胃癌组织切片caveolin1  
FITC免疫荧光和量子免疫荧光不同  
时间点对比

Olympus BX51, DP72, ×400  
分析软件: CRi多光谱成像系统





# 量子点免疫荧光结果可长期保存

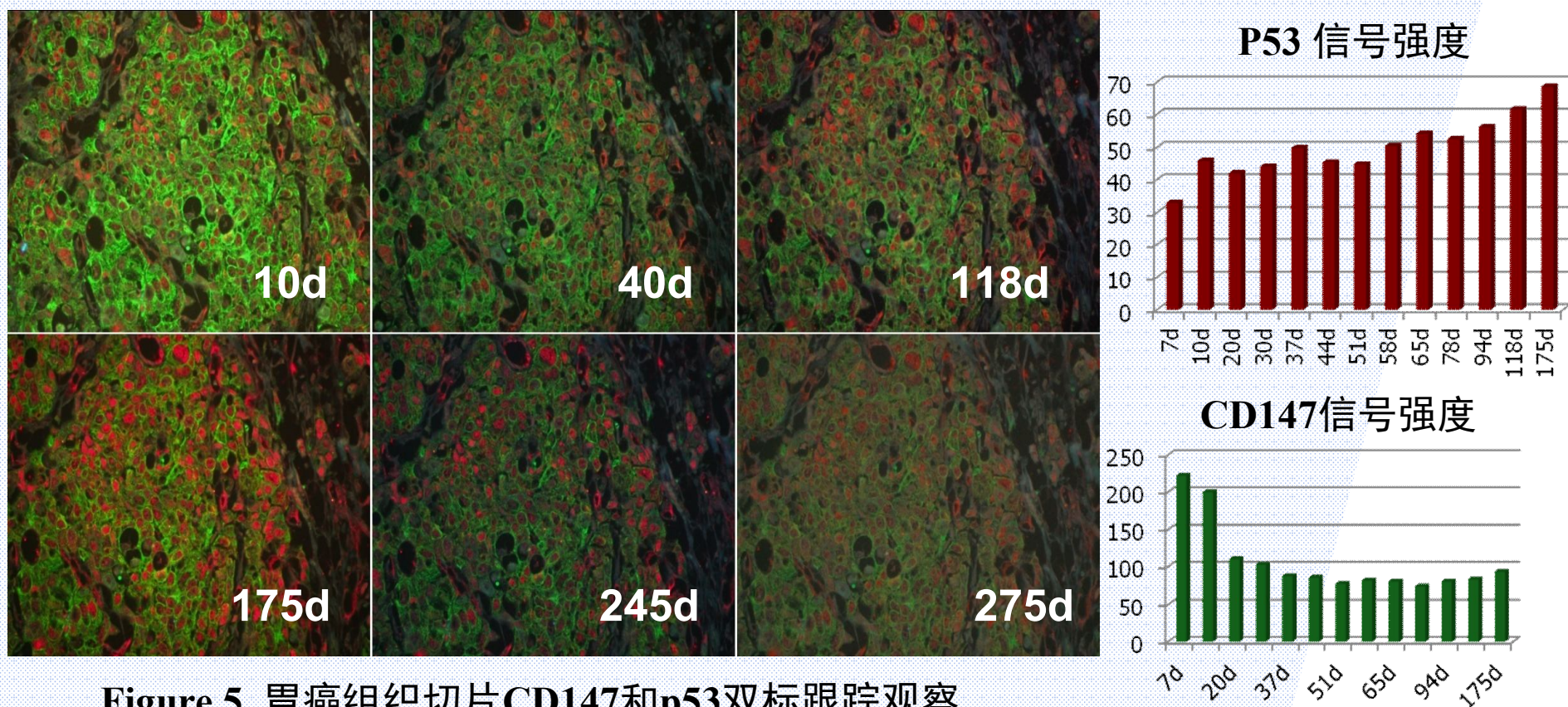


Figure 5. 胃癌组织切片CD147和p53双标跟踪观察  
(Olympus BX51, DP72,  $\times 200$ )



# 量子点免疫荧光可实现定量检测

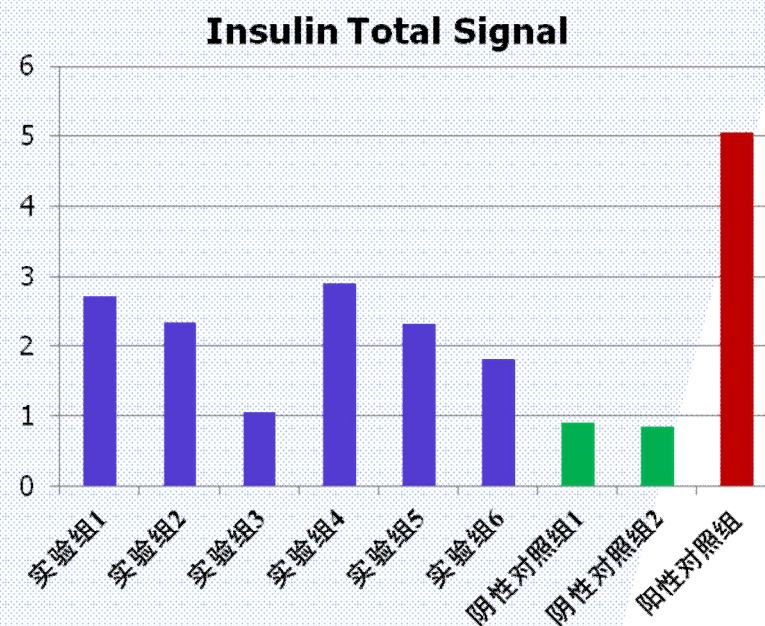
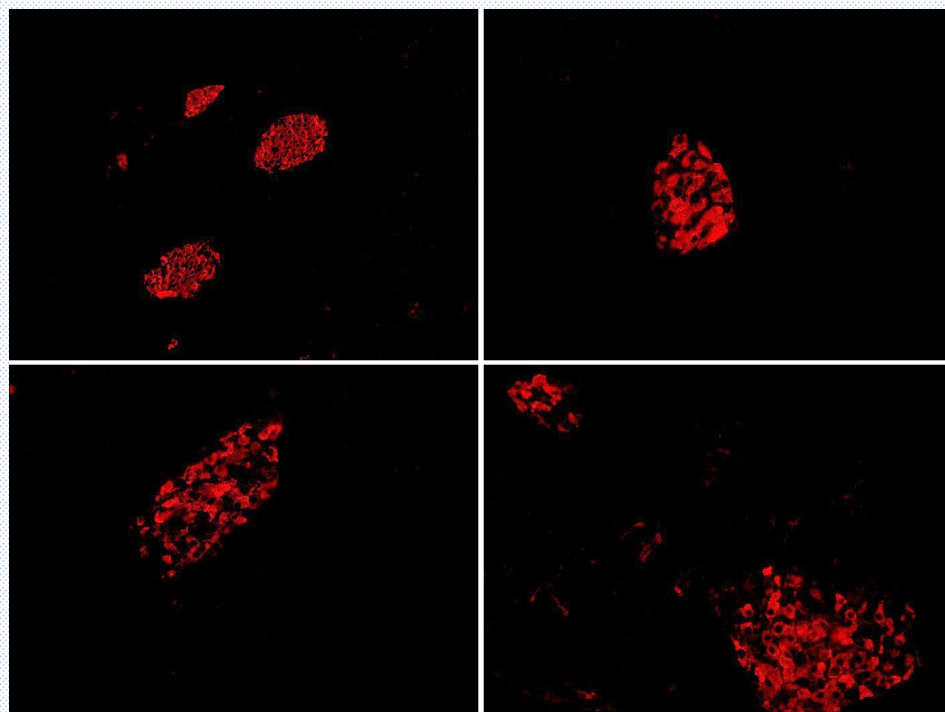


Figure 6. 不同处理组小鼠胰腺组织insulin表达

采集系统: Olympus BX51 荧光显微镜

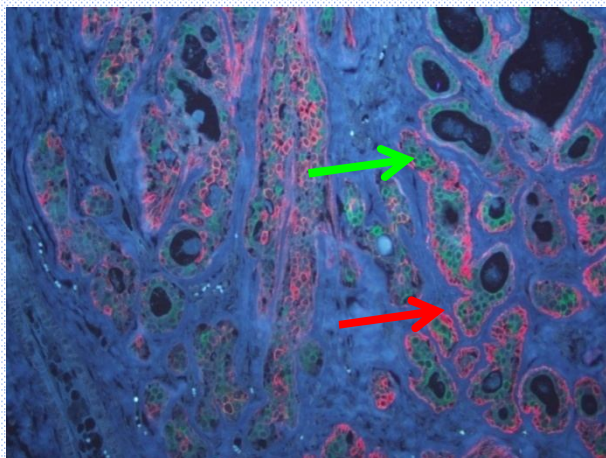
成像及分析系统: CRI公司多光谱成像系统

放大倍数:  $\times 200$

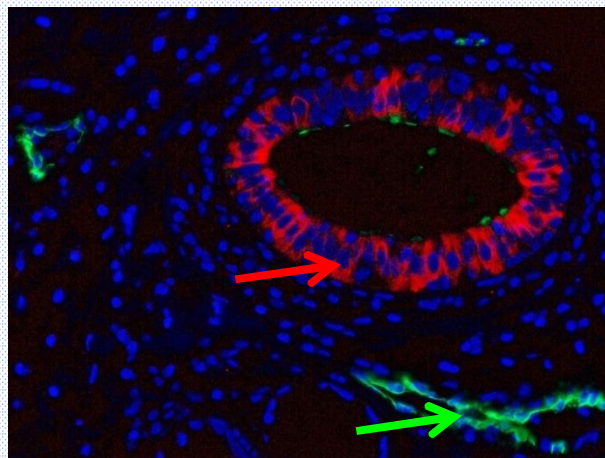
相关文献: *Biomaterials*, 2010: 1-8.



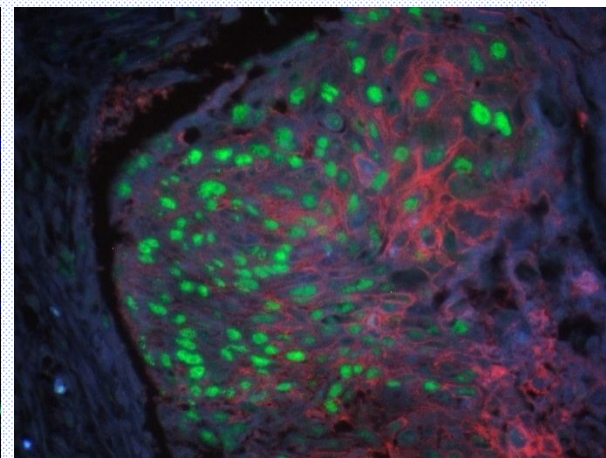
# 量子点免疫荧光可实现多种颜色同时成像



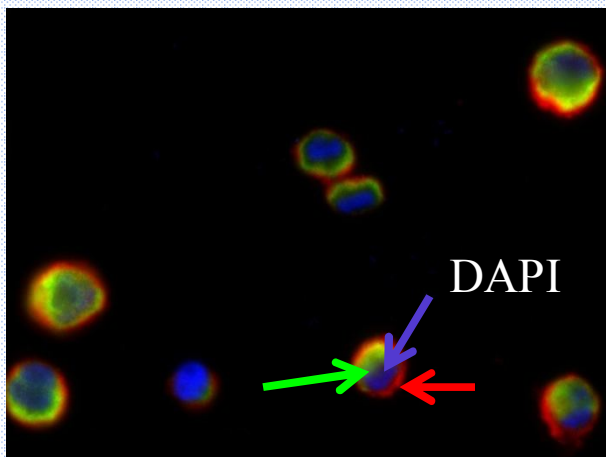
胃癌 CK5&6+CK8&18



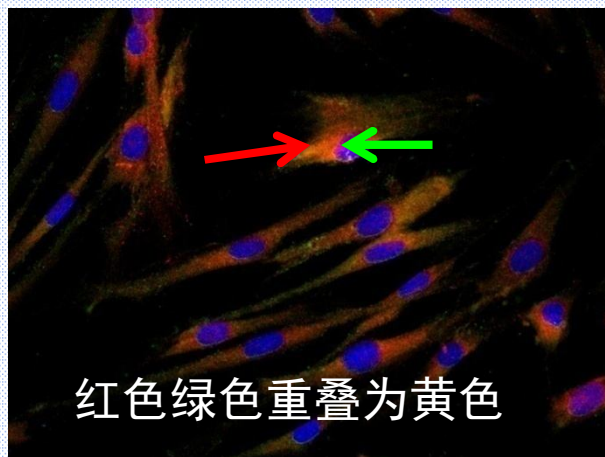
牙齿 CK+MECA-79



胃癌 CK+PCNA

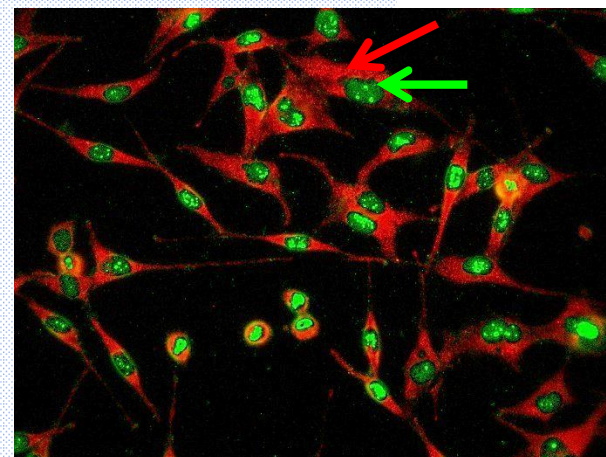


细胞涂片量子点双标



红色绿色重叠为黄色

细胞爬片胞浆双标



细胞爬片胞浆胞核双标



# 量子点免疫荧光可实现抗原表达共定位分析

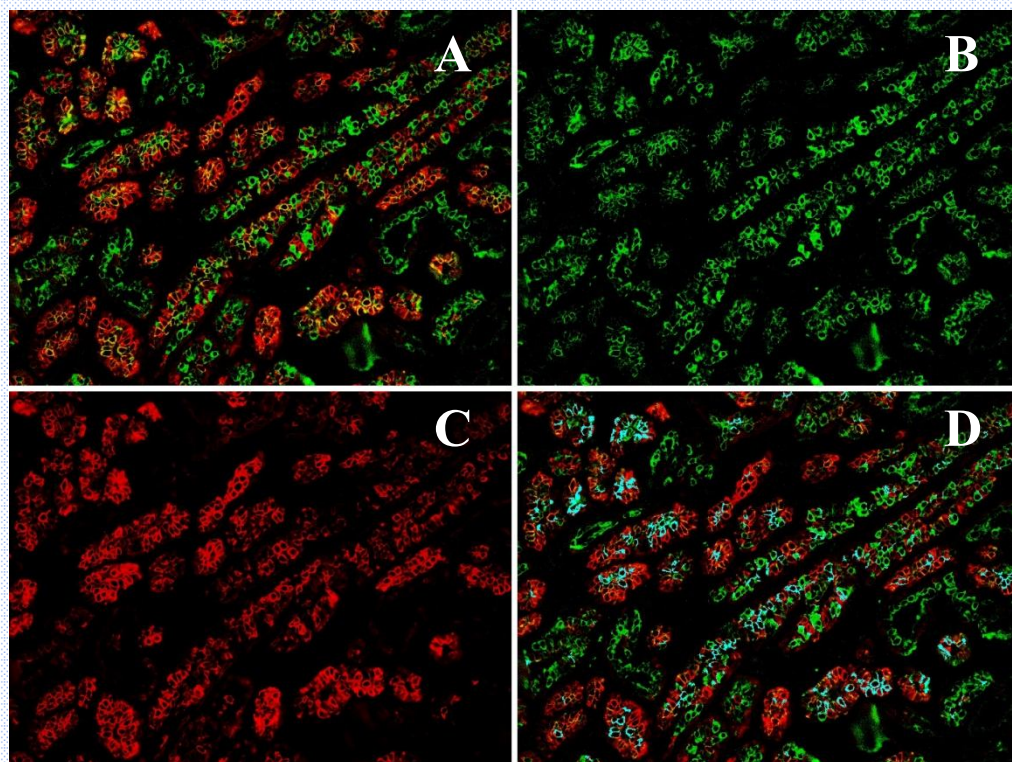


表1: 各指标所占百分比及共定位所占百分比

	% Co-loc.	% Area: CK5&6	% Area: CK8&18
Percent (%)	2.03	15.37	6.56

表2: 各指标定量结果

	Marker Area	Total Signal	Avg Signal	Standard Deviation
CK5&6	55618	3859.33	17.47	30.03
CK8&18	23733	124.28	0.562	1.4

Figure7. 胃癌组织CK5&6和CK8&18双染结果

A: CK5&6 +CK8&18 ;

B: CK5&6; C: CK8&18; D: 共定位图

(红色: 605nm QDs; 绿色: 545nm QDs; 黑色: 背景; 蓝色: 显示两者共表达部位)

荧光显微镜: Olympus BX51

采集系统: 多光谱成像系统

分析系统: 多光谱成像系统

放大倍数: ×200

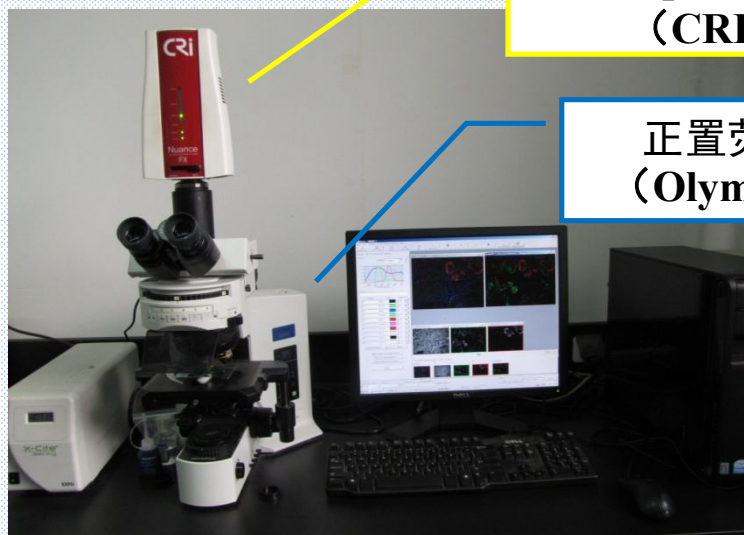
激发条件: 紫外光



# 珈源量子点结合多光谱成像系统的实际应用

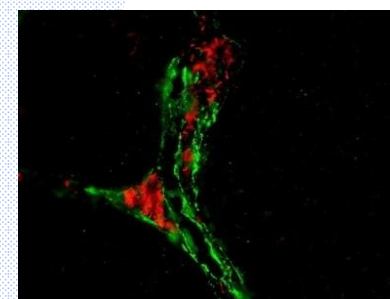
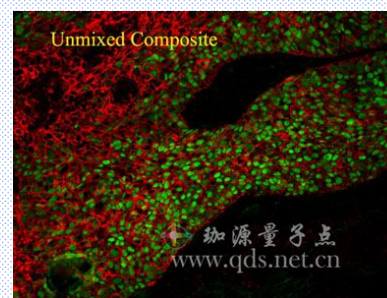
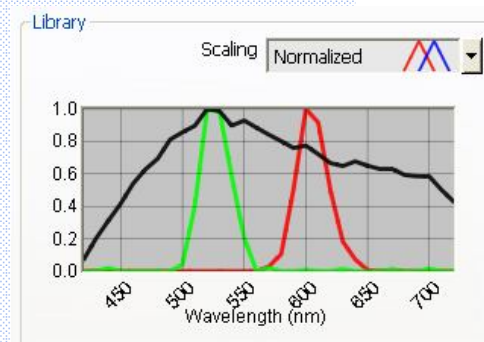
珈源公司多光谱成像系统，可检测420nm到720nm区间任意波段的荧光信号，结合本公司的量子点荧光标记试剂可实现同一样本中多色荧光的同时成像。

- ▶完全去除自发荧光，提高信噪比；
- ▶可分离多种信号，便于分析多种蛋白表达，尤其是不同蛋白的共定位；
- ▶定量分析（包括不同蛋白表达的信号强度、不同蛋白共定位的百分比等）



**Multispectral imaging system  
(CRI, Nuance Fx)**

**正置荧光显微镜  
(Olympus BX51)**



# 胃癌组织切片CD147（胞膜）PCNA（胞核）双标结果

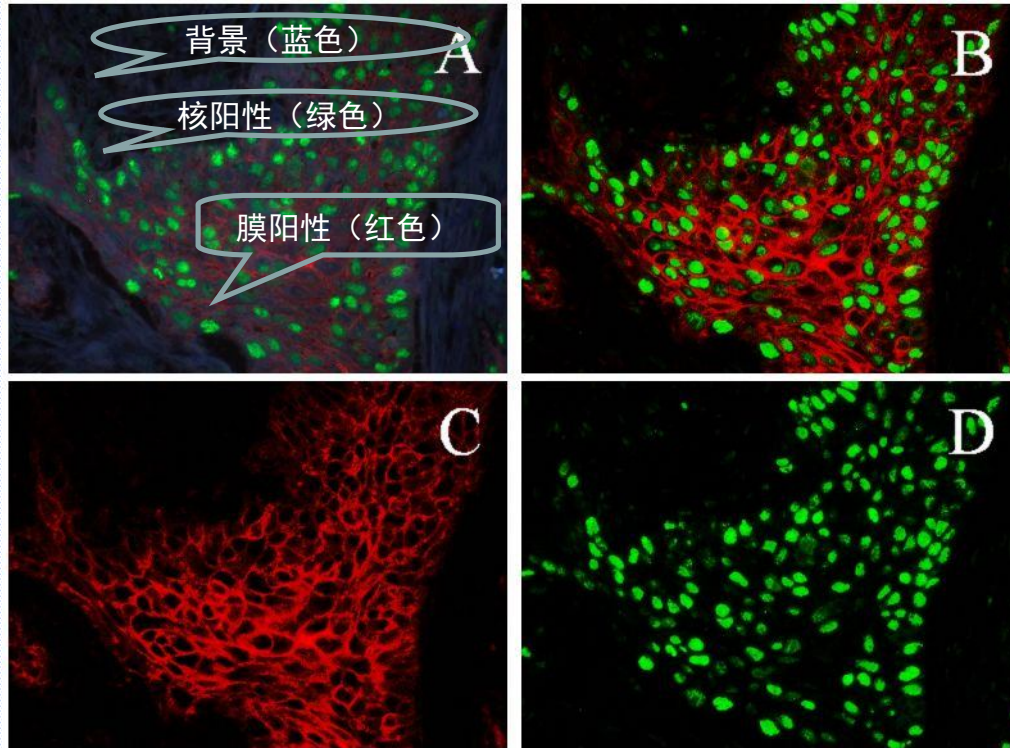


表3：各指标所占百分比及共定位所占百分比

Percent (%)	% Co-loc.	% Area: CD147	% Area: PCNA
	1.84	26.64	11.41

表4：各指标定量结果

Marker	Total Area	Avg Signal	Standard Deviation
CD147	96402	686489.06	1.9
PCNA	41301	879382.19	2.43

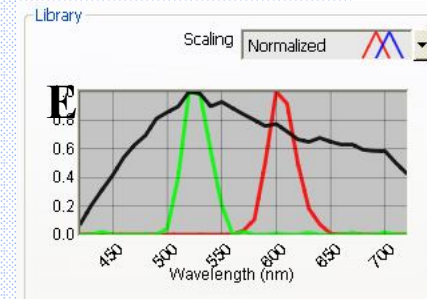


Figure 8. 胃癌组织CD147和PCNA量子点免疫荧光双染结果

A: 原始拍摄图； B: 光谱分离图；

C: 分离CD147； D: 分离PCNA； E: 光谱图

(红色： 605nm QDs； 绿色： 545nm QDs； 黑色： 背景)

# 乳腺癌组织切片HER2（胞膜）CK（胞浆）双标结果

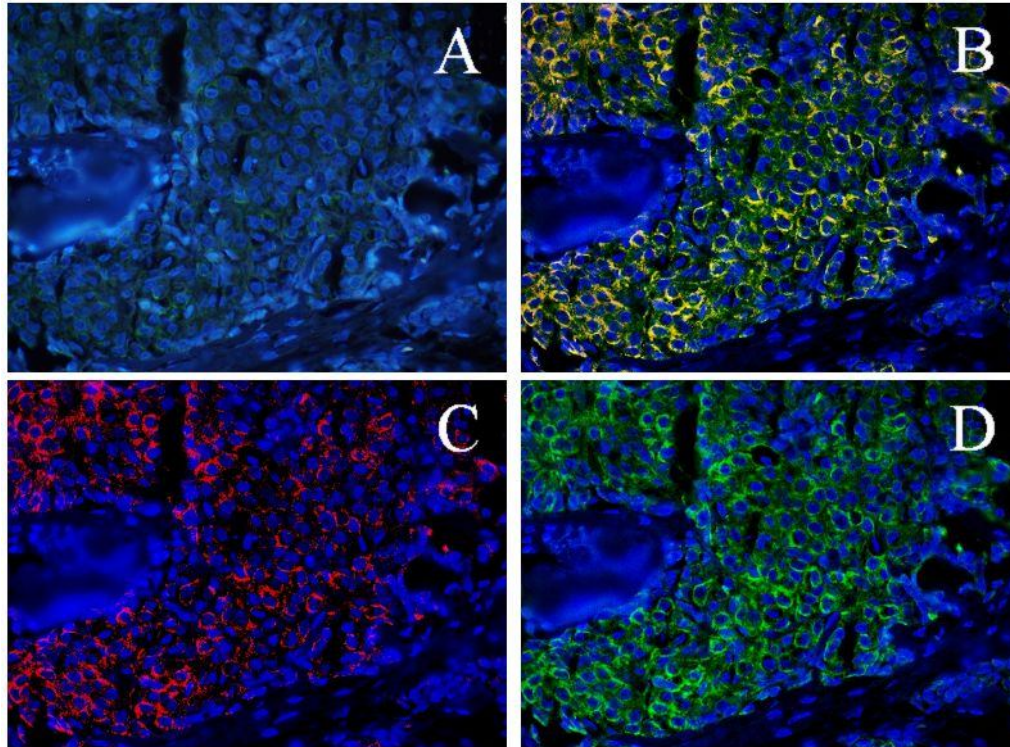


表5: 各指标所占百分比及共定位所占百分比

Percent (%)	% Co-loc.	% Area: CK	% Area: HER2
	4.91	4.93	25.77

表6: 各指标定量结果

Marker	Area	Total Signal	Avg Signal	Standard Deviation
CK	17850	117615.48	0.325	0.89
HER2	93274	1130477	3.12	3.31
DAPI	106854	4528465.5	12.51	12.26

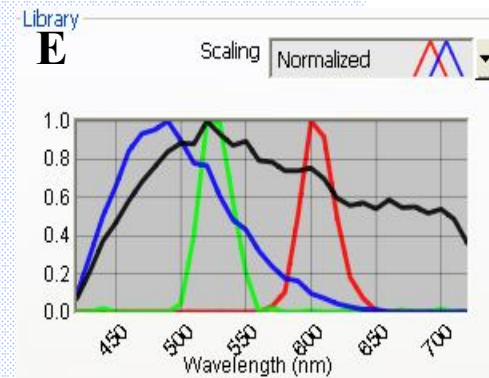
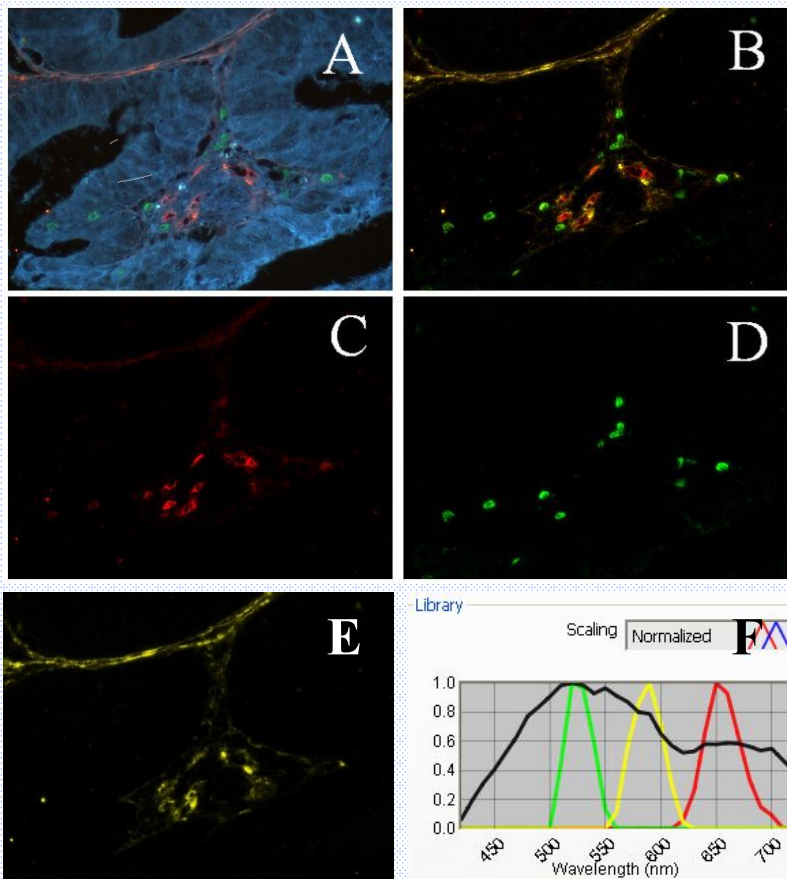


Figure 9. 乳腺癌组织CK和HER2量子点免疫荧光双染结果

A: 原始拍摄图; B: 光谱分离图;

C: 分离CK; D: 分离HER2; E: 光谱图  
(红色: 605nm QDs; 绿色: 545nm QDs;  
蓝色: DAPI; 黑色: 背景)

# 量子点免疫荧光多色标记（胞膜+胞浆+胞核）



**Figure 10.** 量子点免疫荧光三标结果  
 A: 原始拍摄图; B: 光谱分离图; CDE: 分离单色图;  
 F: 光谱图  
 (黑色: 背景; 红色: 655nmQDs;  
 绿色: 525nmQDs; 黄色: 585nmQDs; )

表7: 各指标所占百分比及共定位所占百分比

% Co-loc.	% Area: 指标1	% Area: 指标2	% Area: 指标3
0.1	8.53	3.7	6.85

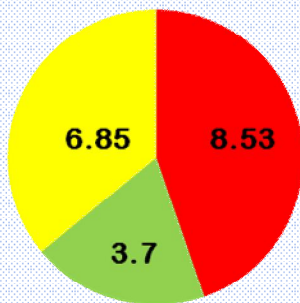
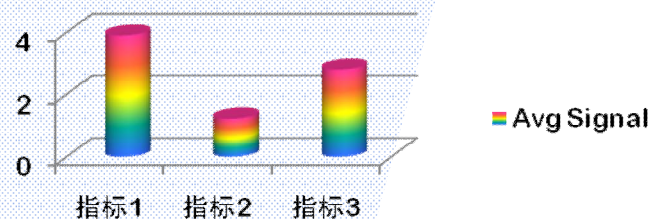


表8: 各指标定量结果

Marker	Total Area	Total Signal	Avg Signal	Standard Deviation
指标1	30874	1415216.63	3.91	10.63
指标2	13387	448497.56	1.24	4.2
指标3	24786	1020498.19	2.82	7.89

Avg Signal



发表文章: . *Biomaterials* 2011, 32 , 2907-2917. (合作单位: 中南医院)





## 使用珈源量子点免疫荧光试剂盒发表的部分文章

1. Richard J. Quantum Dots Brighten Biological Imaging. *Histochemistry and Cytochemistry* 45 (2011) 201–237.
2. Chen, C., Li, Y. (2010) The quantitative detection of total HER2 load by quantum dots and the identification of a new subtype of breast cancer with different 5-year prognosis, *Biomaterials*.
3. Chen, H. L., (2010) Detection of EBV in nasopharyngeal carcinoma by quantum dot fluorescent in situ hybridization, *Exp Mol Pathol* 89, 367-371.
4. He, Y., Li, Y., (2011) In Situ Spectral Imaging of Marker Proteins in Gastric Cancer with Near-infrared and Visible Quantum Dots Probes, *Talanta*.
5. Peng, C. W., Li, Y. (2011) Patterns of cancer invasion revealed by QDs-based quantitative multiplexed imaging of tumor microenvironment, *Biomaterials*.



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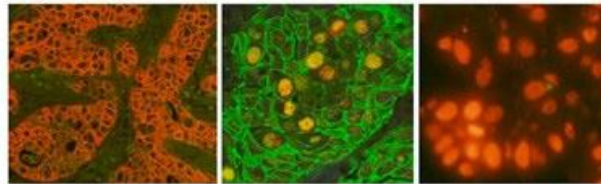
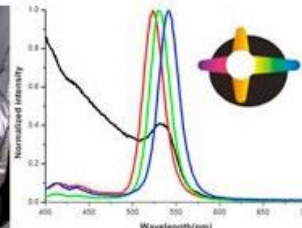
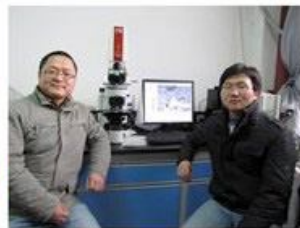
- ▶ [Wuhan Jiayuan Quantum Dots](#)
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Mar 16, 2010

#### Quantum dots highlight tumour heterogeneity

Breast cancer (BC) is the number one cancer killer in women. BC is a heterogeneous tumour, and a good understanding of such heterogeneity is essential in order to optimize the treatment strategy. Quantum dot-based immunofluorescent nanotechnology (QD-IHC) for molecular pathology has potential advantages in delineating the tumour heterogeneity.



Quantum-dot imaging applied to breast-cancer cells

Researchers at Wuhan University, China, have developed very sensitive and specific QD-IHC technology to image HER2 and

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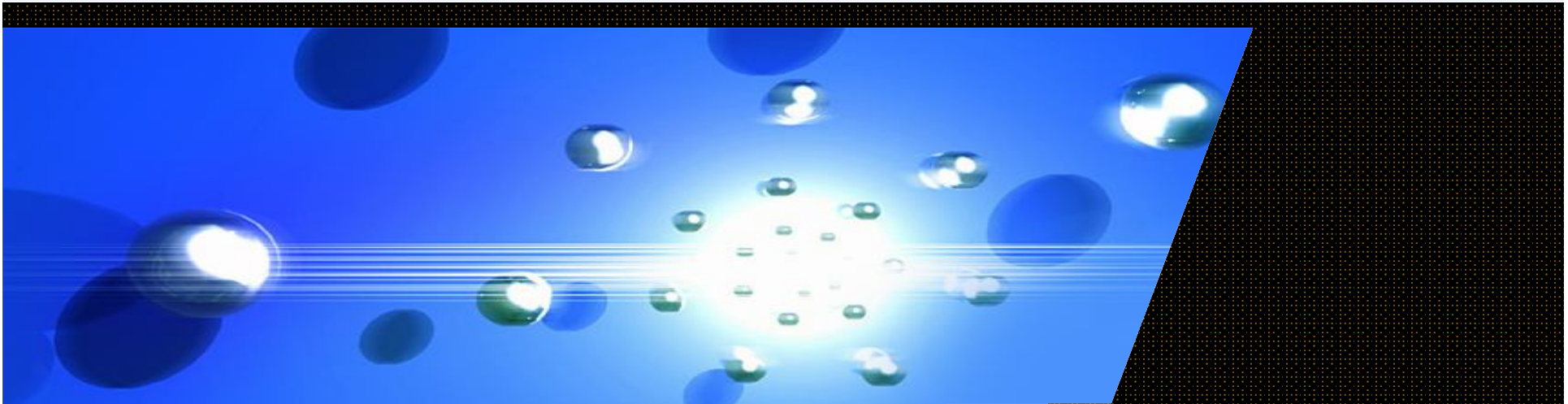
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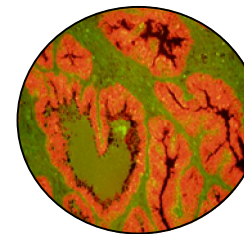
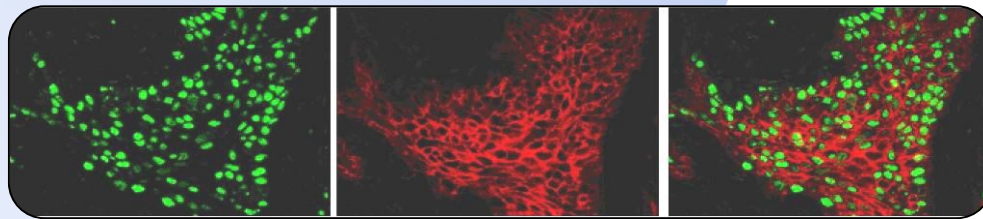
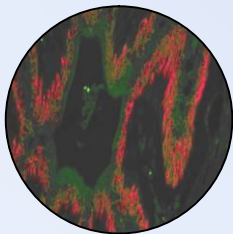
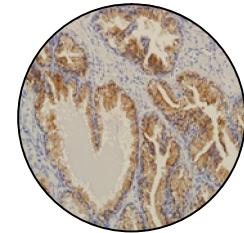
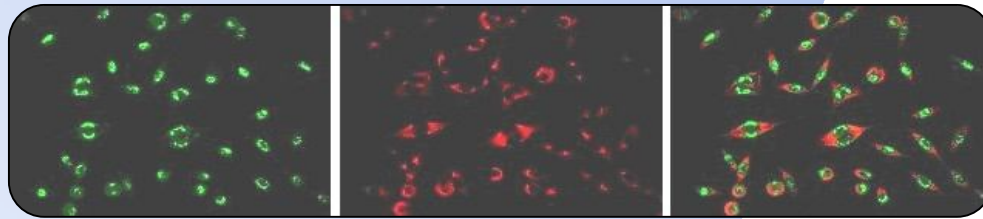
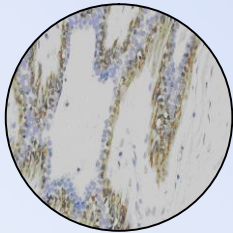
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Thank You !



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