

Immunhisztokémia

2011. / 5

Immunológiai és Biotechnológiai Intézet

PTE KK

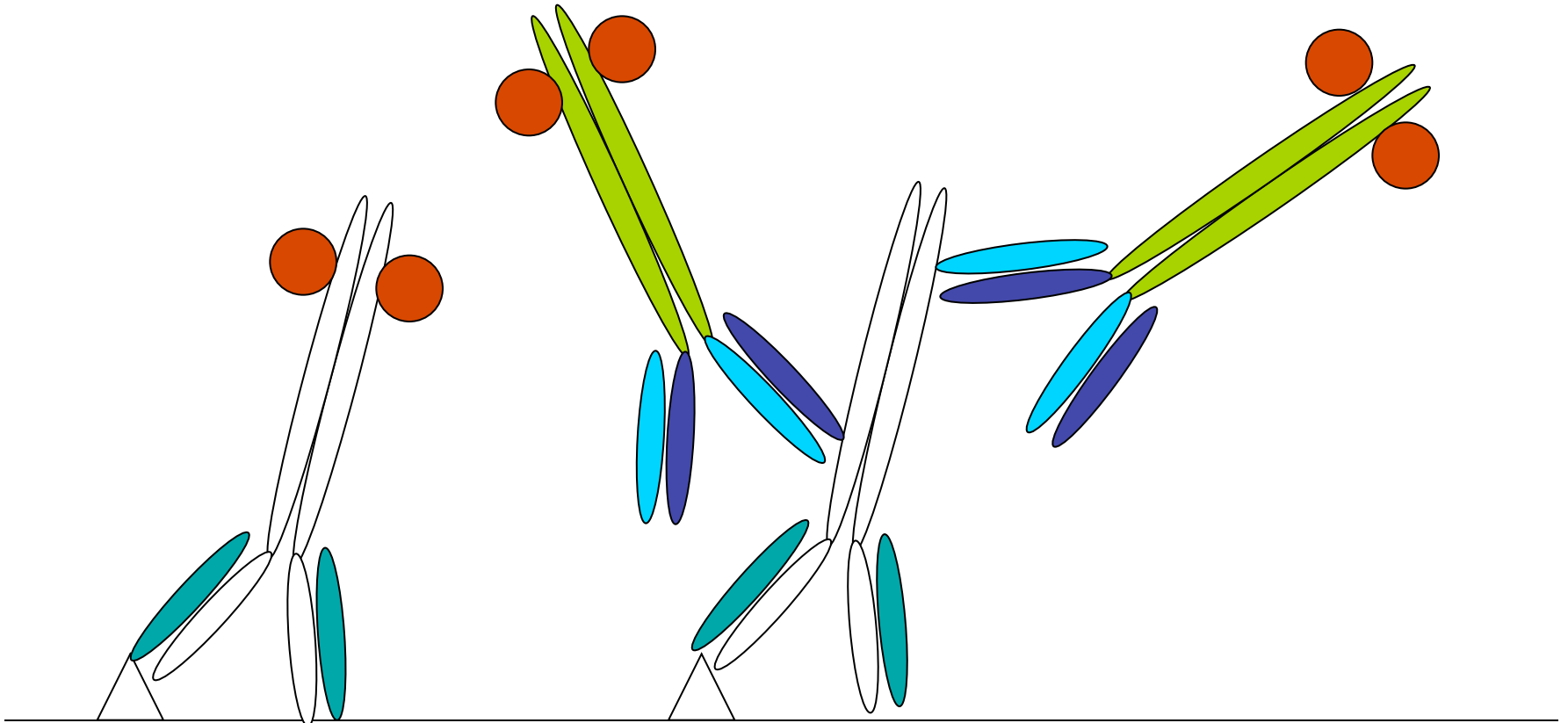
Direkt immunhisztokémia

1. **Fixálás acetonban**
2. **Endogén peroxidáz aktivitás gátlása fenilhidrazinnal (PBS-ben oldva) 10 min.**
3. **Mosás PBS-sel 2 x 2 min**
4. **Nemspecifikus protein-kötőhelyek blokkolása 5 % BSA-PBS-sel 10 min**
5. **Inkubálás peroxidáz (HRPO) konjugált antitesttel, 30 min**
6. **Mosás, 3 x PBS-sel 2 min**
7. **Előhívás szubsztrát oldattal (amino-ethyl carbasol (AEC) + H₂O₂ 0.1 M Na-acetát pufferben (pH 5.2))**

Direkt, indirekt immunhisztokémia

▲ : antigén

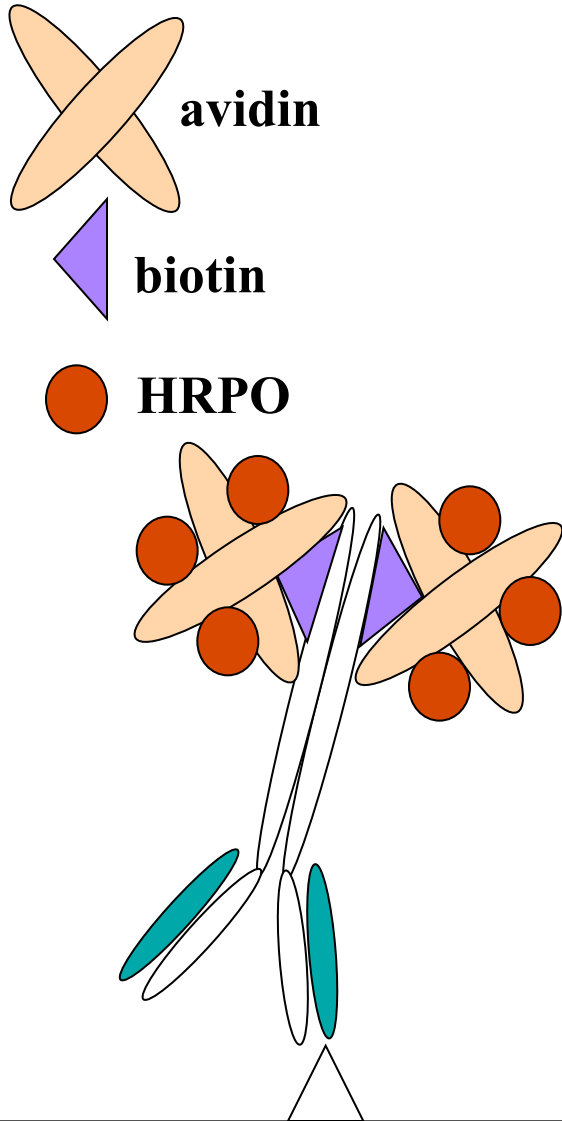
● : enzime (HRPO, ALP), fluorochrome (FITC, TRITC, PE)



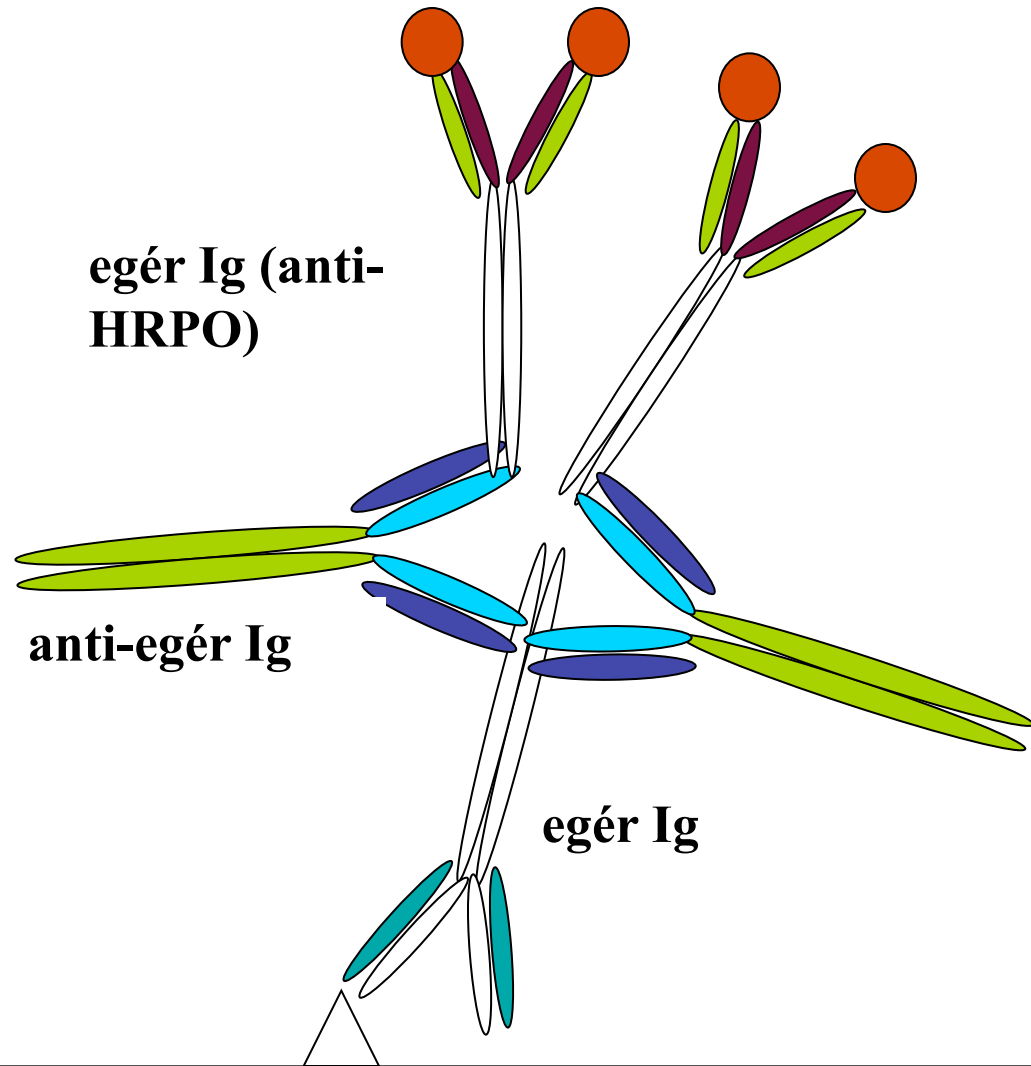
Direkt módszer

Indirekt módszer

Komplex előhívó rendszerek



**(Strept)avidin-biotin-
HRPO komplex**

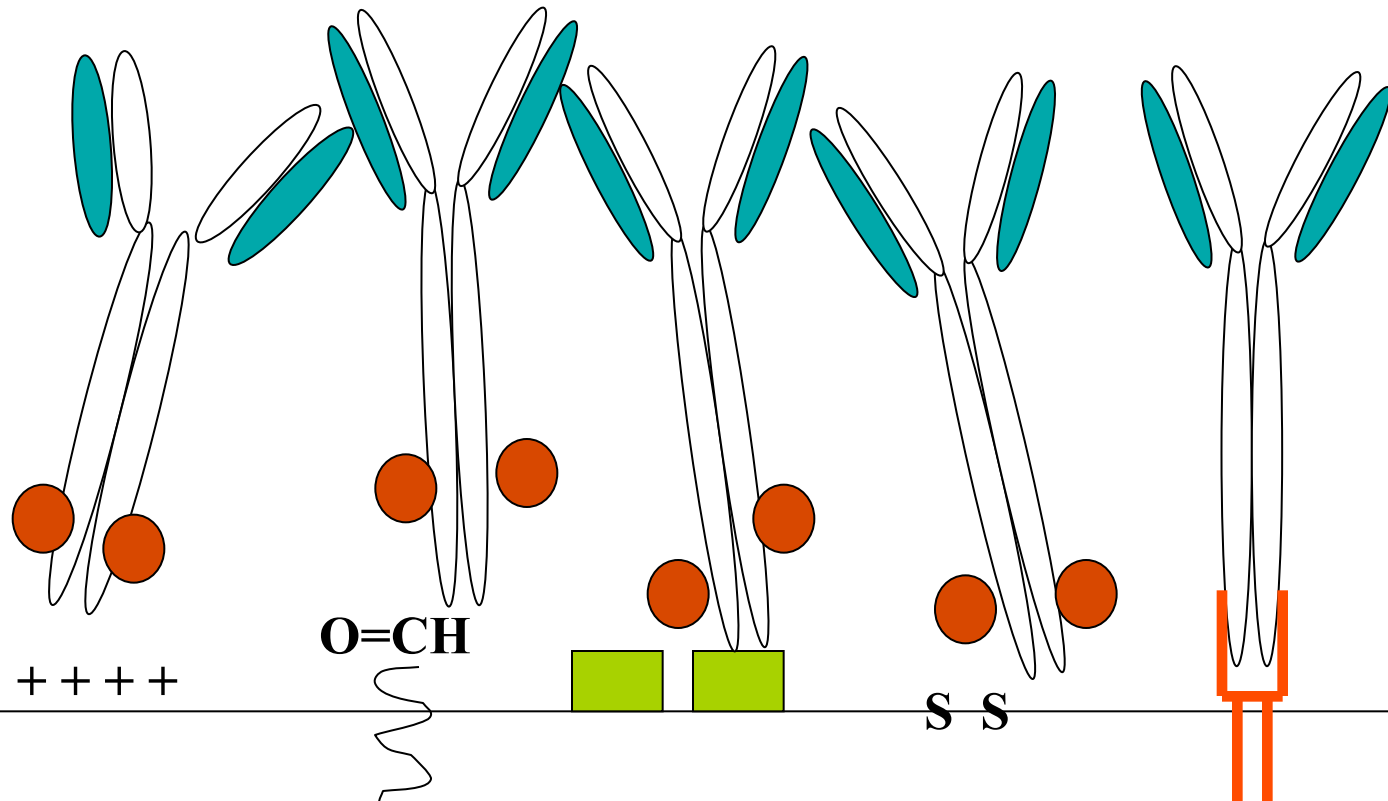
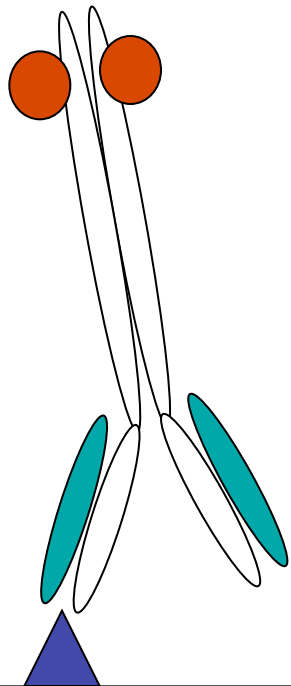


**Peroxidáz-antiperoxidáz
komplex**

Specifikus és nemspecifikus interakciók a szövetek és az ellenanyagok között

Specifikus Ag-
At kapcsolódás

Nemspecifikus Ag-At kapcsolódás



Ionos

Aldehyd

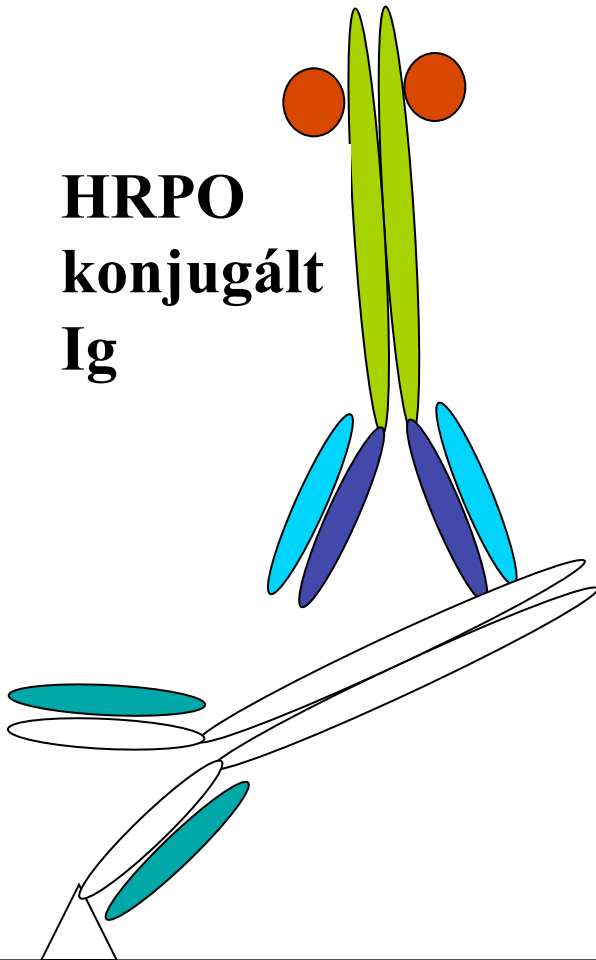
Hidrofób

Szulfid

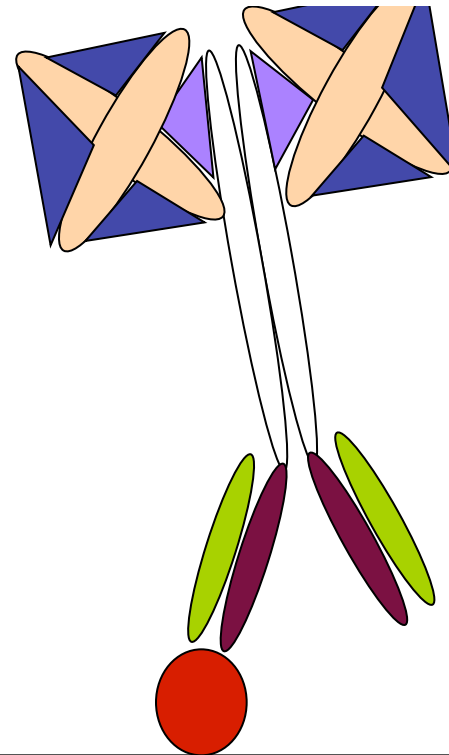
Fc-receptor

Kettős jelölés

HRPO
konjugált
Ig



(Strept)avidin-
biotin AP komplex



Ag 1

Ag 2

A leggyakoribb enzim-szubsztrát rendszerek

Peroxidáz (HRPO)

DAB – diaminobenzidin (barna)

AEC – amino-ethyl-carbasol (vörös)

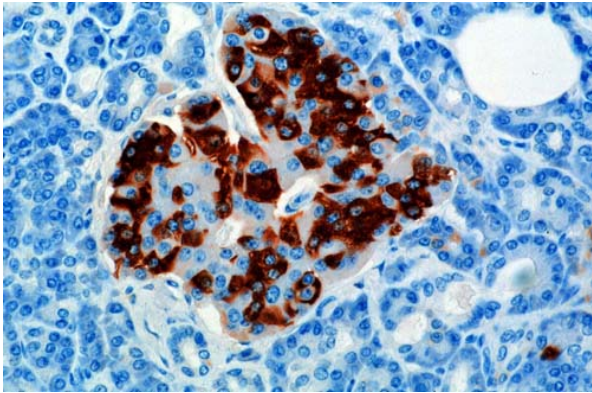
True Blue (kék)

Alkalikus Foszfátáz (ALP)

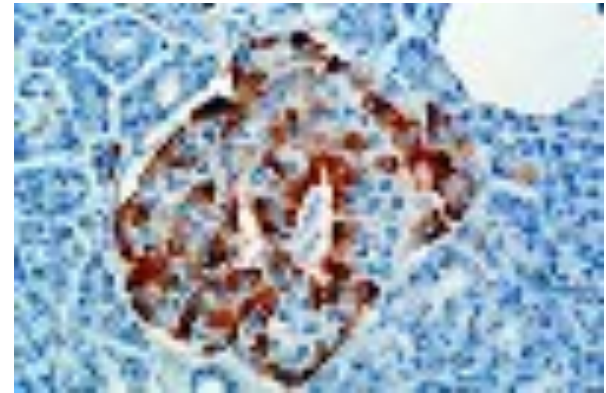
NBT – nitroblue tetrasolium (kék)

BCIP – bromo-chloro-indoyl foszfát (kék)

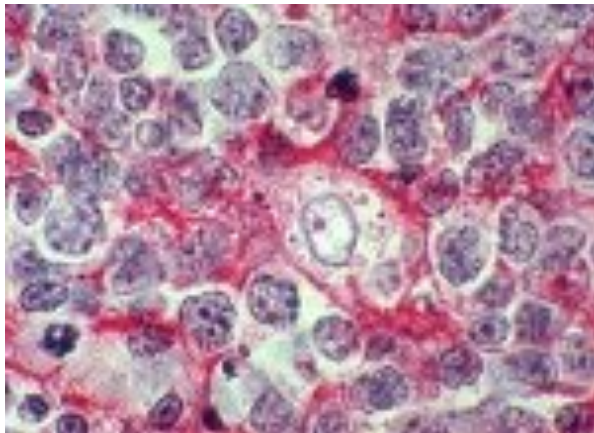
Immunhisztokémia



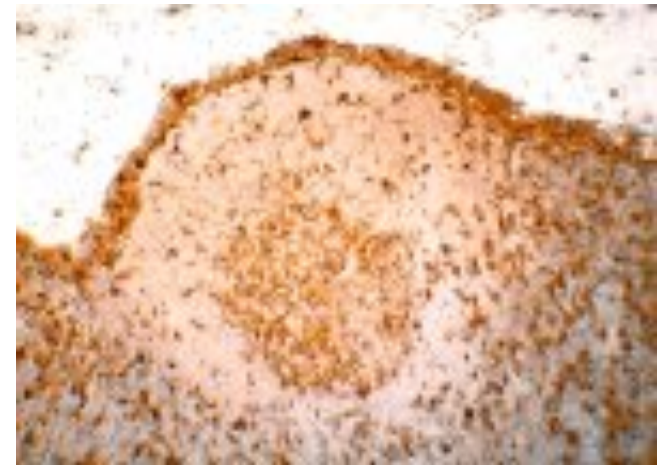
Pancreas, anti-insulin



Pancreas, anti-glukagon



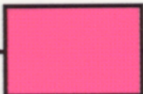

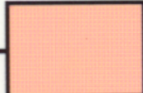


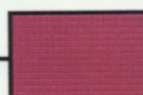


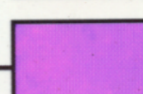


FDC anti-CD21



DC anti-p55 (nyirokcsomó)

Szubsztrát rendszerek

Enzyme Label	Substrate System	Color Reaction	End Product	Application
Alkalien Phosphatase	p-Nitrophenyl Phosphate (pNPP)		Soluble	ELISA
	5-Bromo-4-Chloro-3-Indolyl Phosphate/ Nitro Blue Tetrazolium (BICP/NBT)		Insoluble	Immunoblotting Immunohistology
	Fast Red/Naphtanol AS-TR Phosphate		Insoluble	Immunoblotting Immunohistology
Peroxidase	2,2'-Azino-bis (3-Ethylbenzthiazoline- 6-Sulfonic Acid) (ABTS)		Soluble	ELISA
	o-Phenylenediamine (OPD)		Soluble	ELISA
	3,3',5,5'-Tetramethylbenzidine (TMB)		Soluble	ELISA
	o-Dianisidine		Soluble	ELISA
	5-Aminosalicylic Acid (SAS)		Soluble	ELISA
	3,3'-Diaminobenzidine (DAB)		Insoluble	Immunoblotting Immunohistology
	3-Amino-9-Ethylcarbazole (AEC)		Insoluble	Immunoblotting Immunohistology
	4-Chloro-1-Naphthol (4C1N)		Insoluble	Immunoblotting Immunohistology

Fluorochrome Specifications

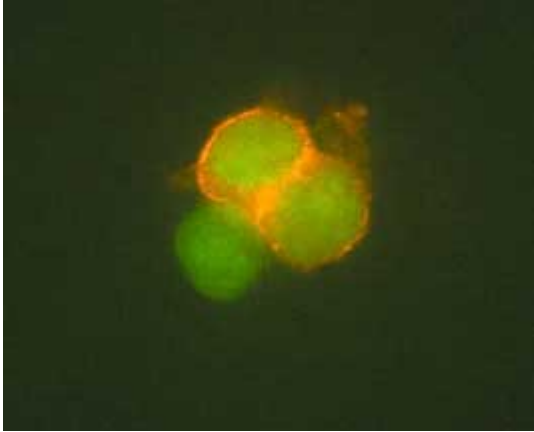
Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)	Em-Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACStar Plus™	BD FACSVantage™ SE	BD™ LSR	BD™ LSR II	BD FACSAria™	BD FACSAria™
Alexa Fluor® 405	Blue	401	360, 405, 407	421				✓		✓	✓	
Pacific Blue®	Blue	410	360, 405, 407	455				✓		✓	✓	
Alexa Fluor® 488	Green	495	488	519	✓	✓	✓	✓	✓	✓	✓	
FITC	Green	494	488	519	✓	✓	✓	✓	✓	✓	✓	
PE	Yellow	496, 546	488, 532	578	✓	✓	✓	✓	✓	✓	✓	✓
PE-Texas Red®	Orange	496, 546	488, 532	615	✓	✓	✓	✓	✓	✓	✓	
Texas Red®**	Orange	595	595	615			✓	✓				
APC*	Red	650	595, 633, 635, 647	660		✓	✓	✓	✓	✓	✓	✓
Alexa Fluor® 647	Red	650	595, 633, 635, 647	668		✓	✓	✓	✓	✓	✓	✓
PE-Cy5*	Red	496, 546	488, 532	667	✓	✓	✓	✓	✓	✓	✓	
PerCP	Red	482	488, 532	678	✓	✓			✓	✓	✓	
PerCP-Cy5.5	Far Red	482	488, 532	695	✓	✓	✓	✓	✓	✓	✓	✓
PE-Cy7	InfraRed [†]	496, 546	488, 532	785	✓	✓	✓	✓	✓	✓	✓	✓
APC-Cy7	InfraRed [†]	650	595, 633, 635, 647	785			✓	✓	✓	✓	✓	✓

[†] *InfraRed* detection requires a Hamamatsu R3896 Photomultiplier Tube (comes with detector option).

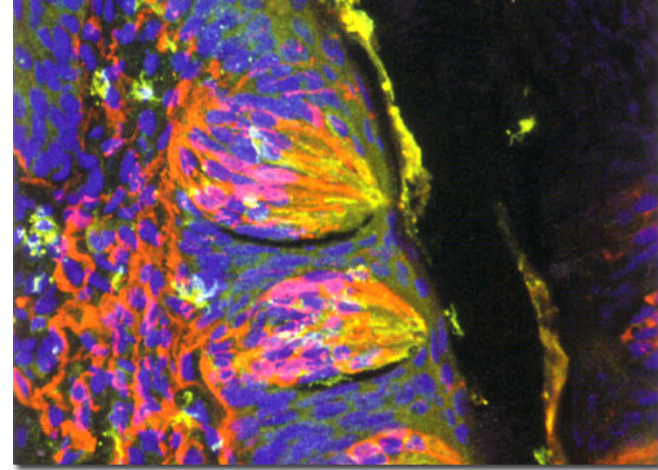
* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

** Texas Red® detection requires a dye laser.

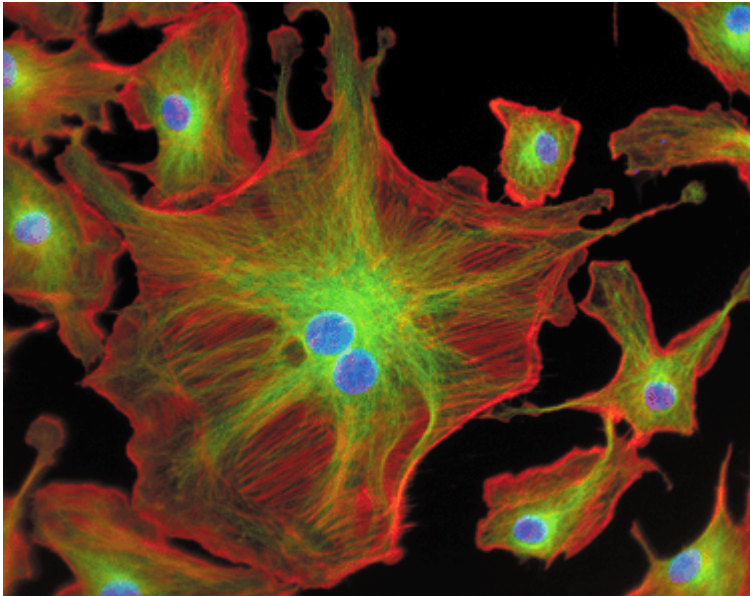
Immunfluoreszcencia



CD4+ T lymphociták



Patkány ízlelőbimbó



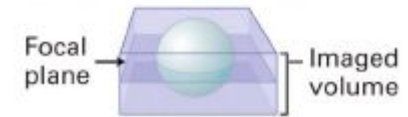
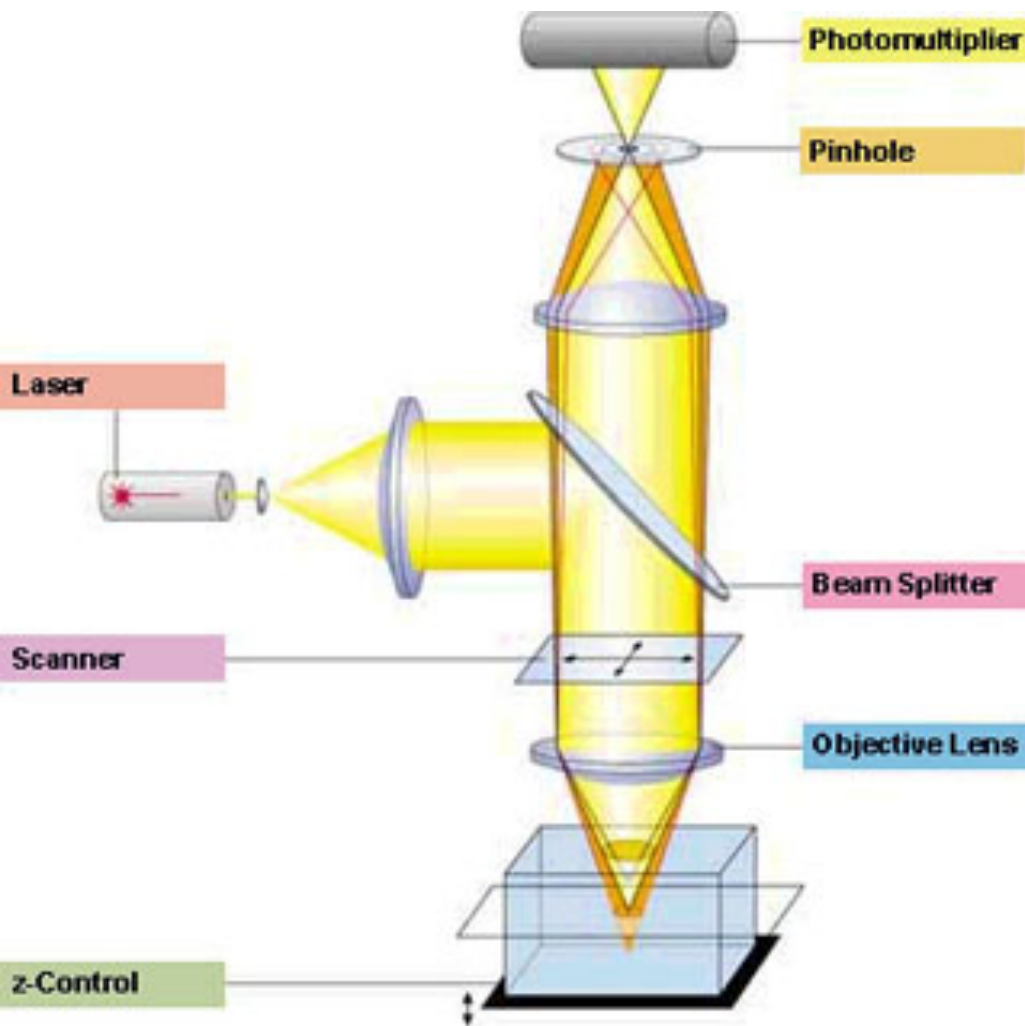
Endothel

tubulin-zöld

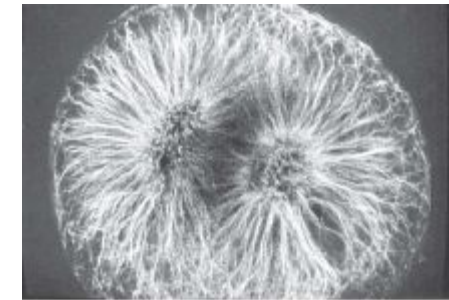
actin-piros

nucleus-kék

Konfokális mikroszkópia

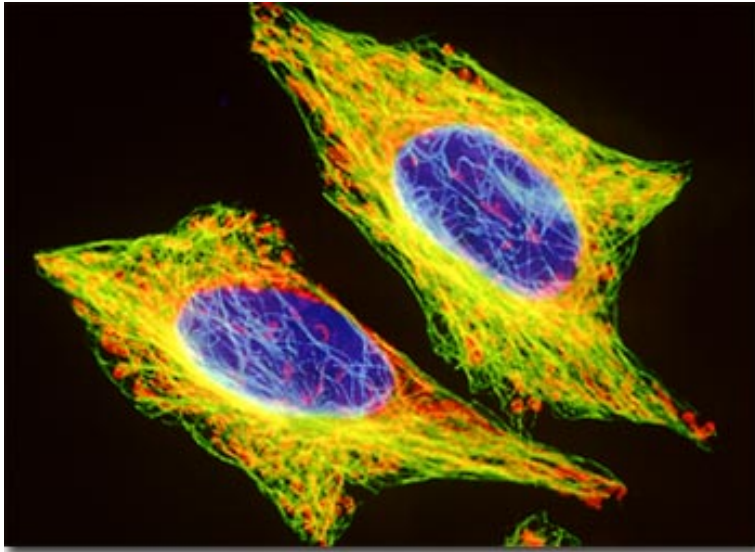


Konvencionális fluoreszcens mikroszkópos kép, anti-tubulin, mitotikus sejt

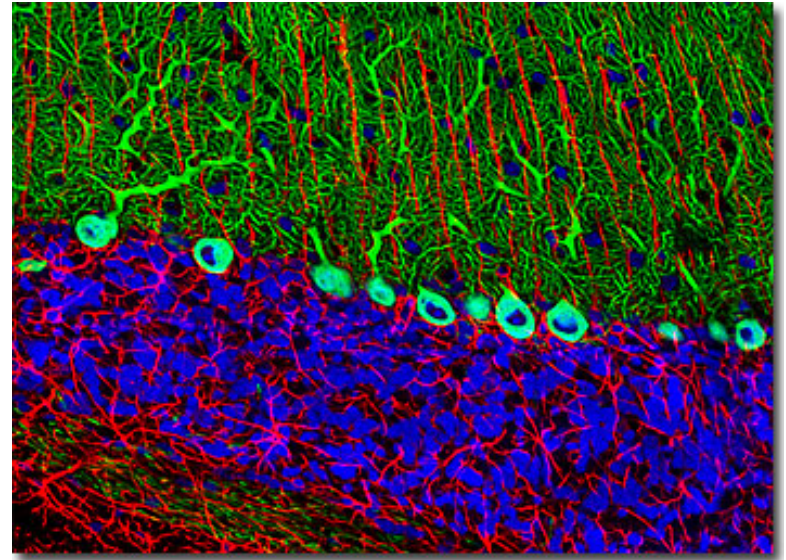


Konfokális mikroszkópos kép

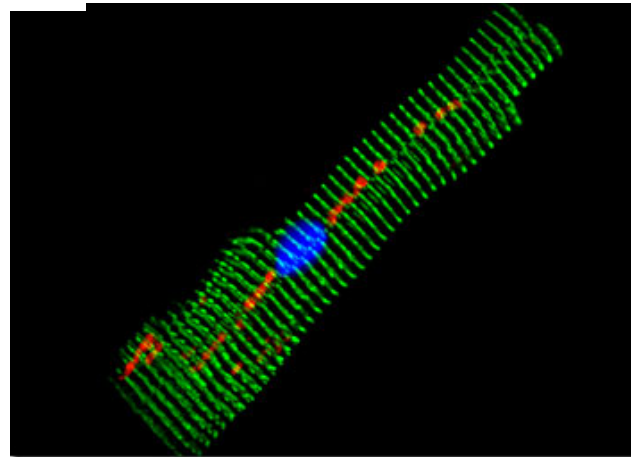
Konfokális mikroszkópia



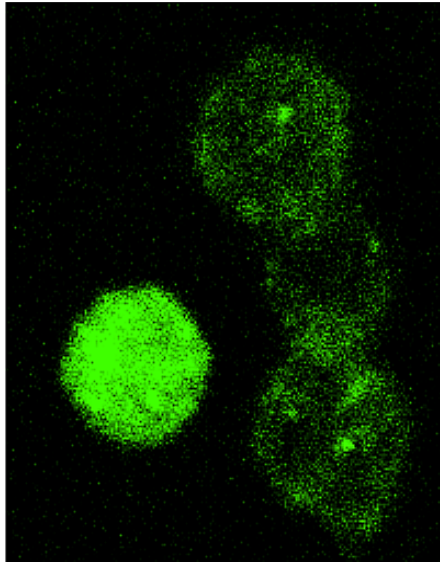
HeLa sejtek



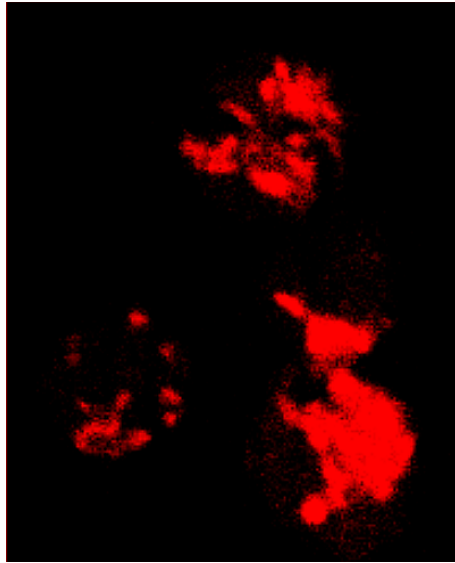
Patkány cerebellum



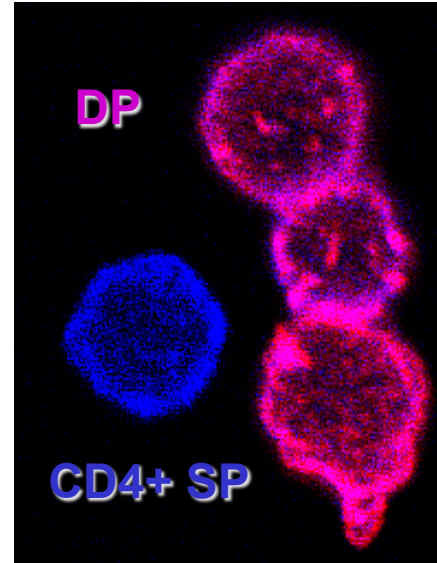
Szívizom



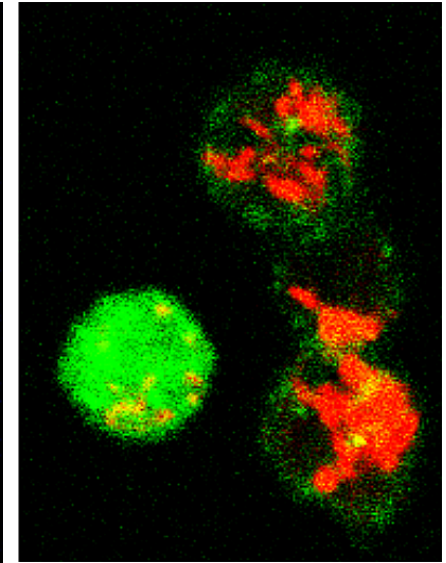
**α -Glucocorticoid
receptor-FITC**



**Chloromethyl-X-
rosamine
(Mitochondrion)**



**α -CD4-Pacific Blue
 α -CD8-Alexa fluor 647**



**Chloromethyl-
X-rosamine –
GR merge**

Lép – immunhisztokémia

Anti-egér Ig-HRPO (AEC)

