Immunhisztokémia

2011. / 5

Immunológiai és Biotechnológiai Intézet

PTE KK

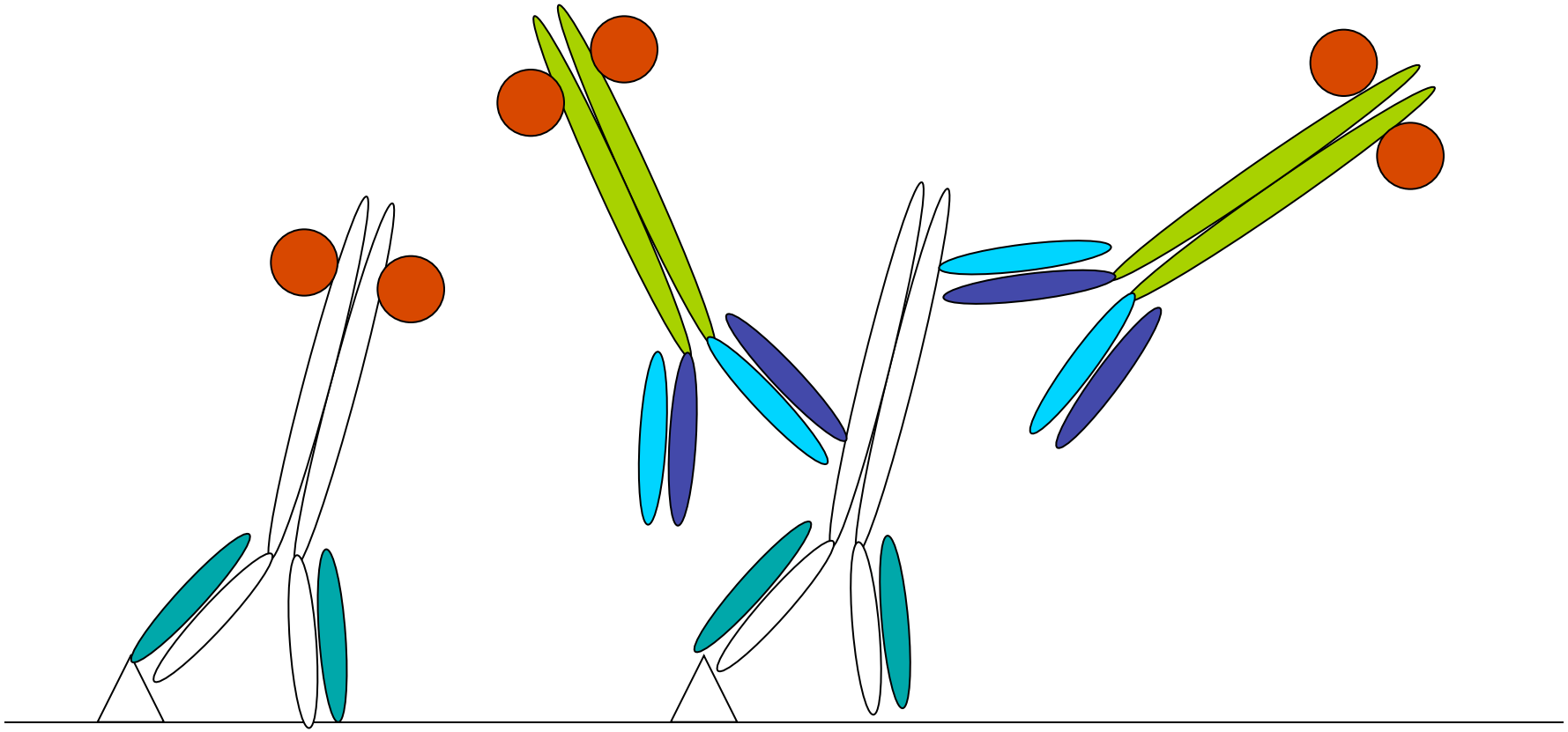
Direkt immunhisztokémia

1. Fixálás acetonban
2. Endogén peroxidáz aktivitás gátlása fenil-hidrazinnal (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) + H_2O_2 0.1 M Na-acetát pufferben (pH 5.2)

Direkt, indirekt immunhisztokémia

: antigén

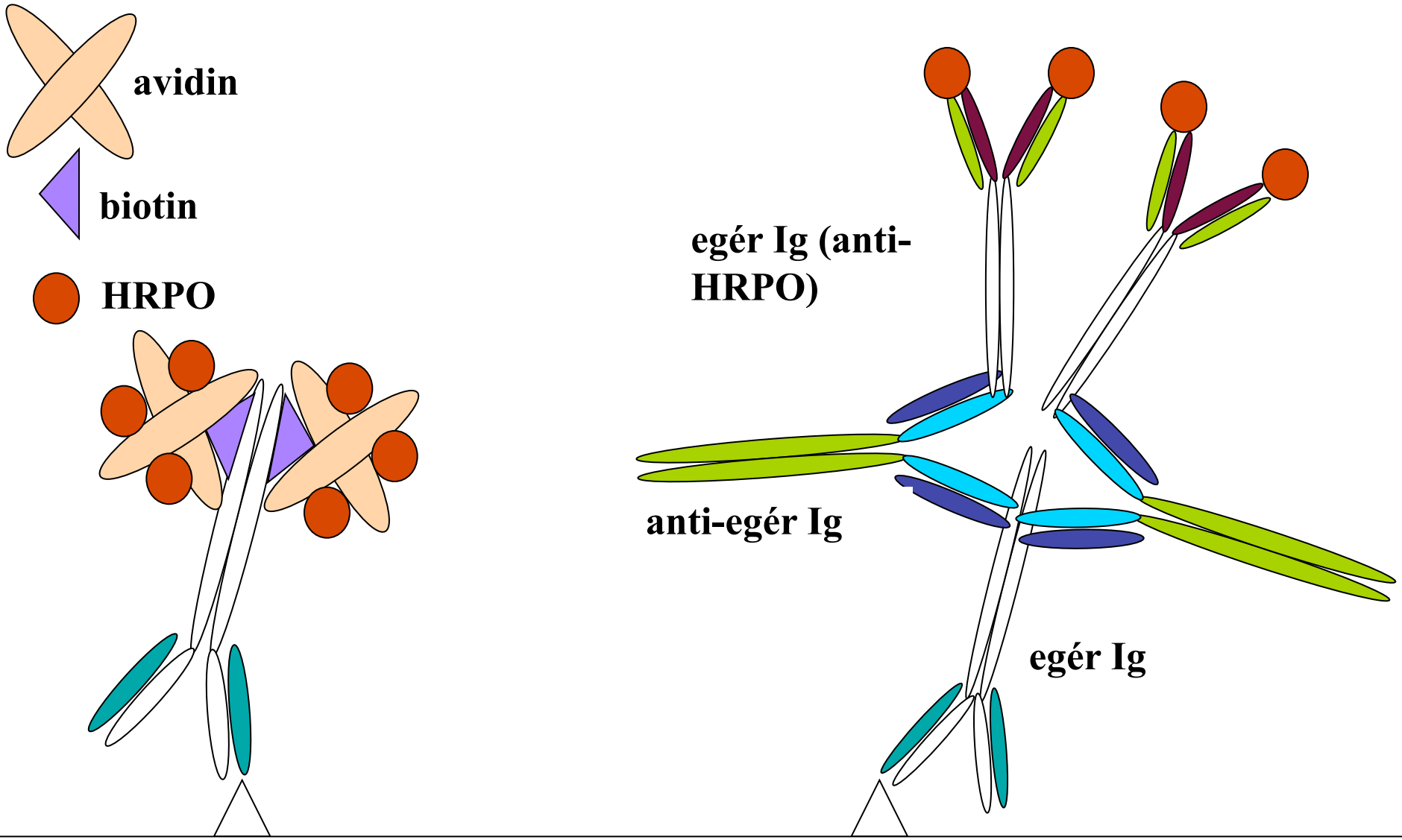
: enzime (HRPO, ALP), fluorochrome (FITC, TRITC, PE)



Direkt módszer

Indirekt módszer

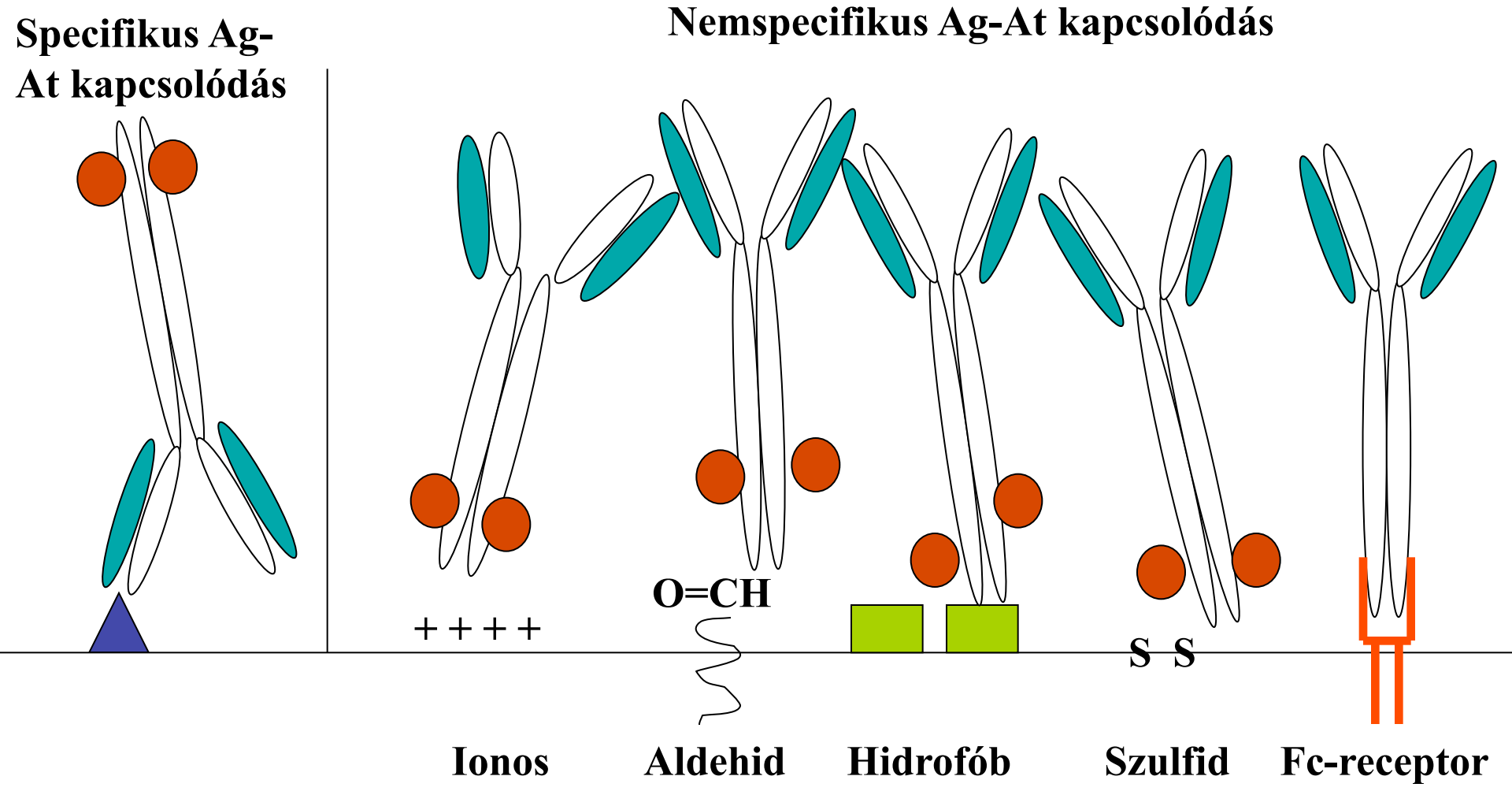
Komplex előhívó rendszerek



(Strept)avidin-biotin-HRPO komplex

Peroxidáz-antiperoxidáz komplex

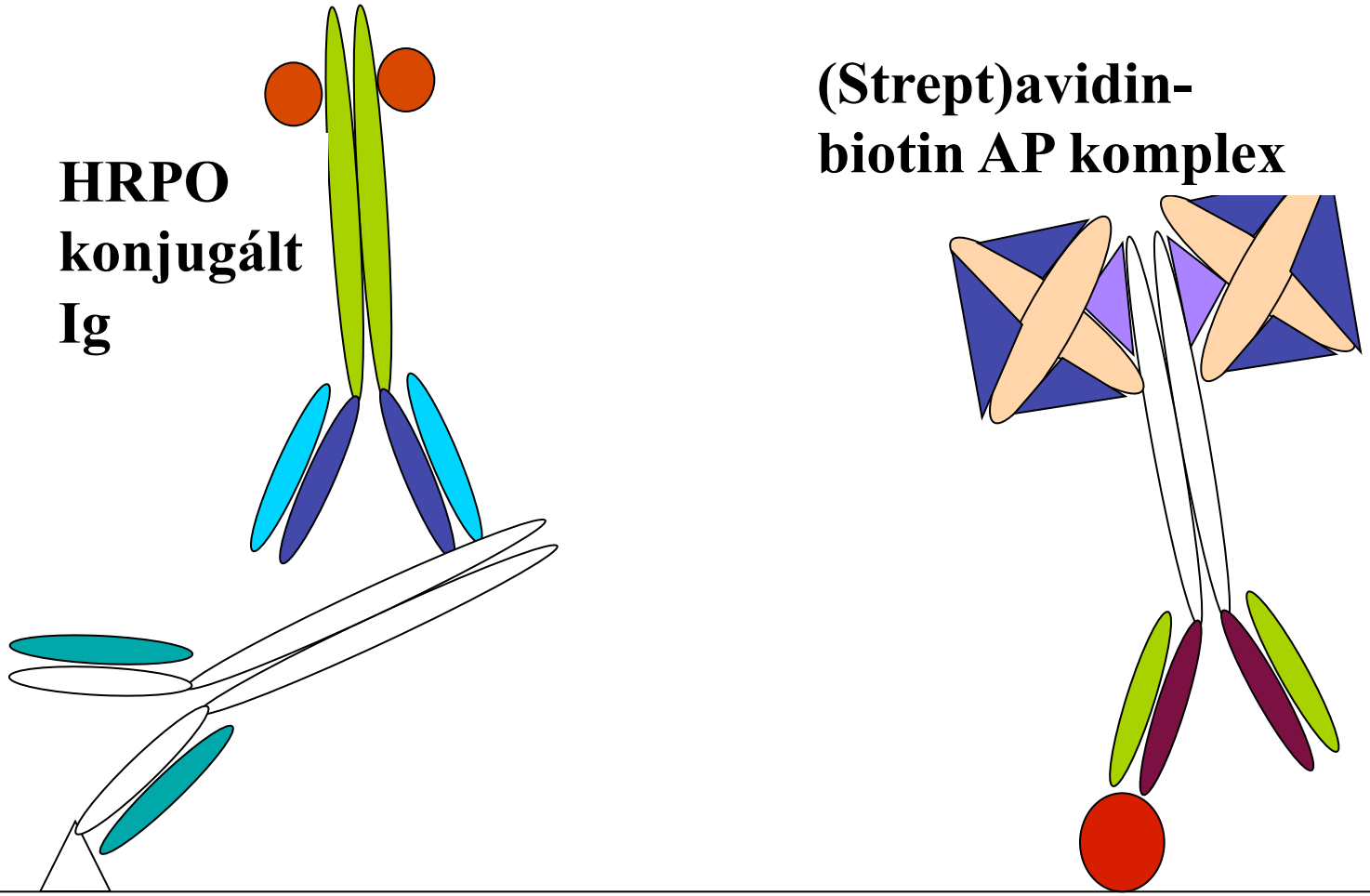
Specifikus és nemspecific interakciók a szövetek és az ellenanyagok között



Kettős jelölés

HRPO konjugált Ig

(Strept)avidin-biotin AP komplex



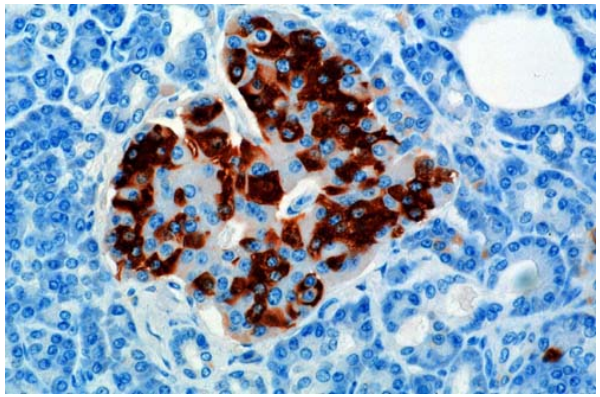
Ag 1

Ag 2

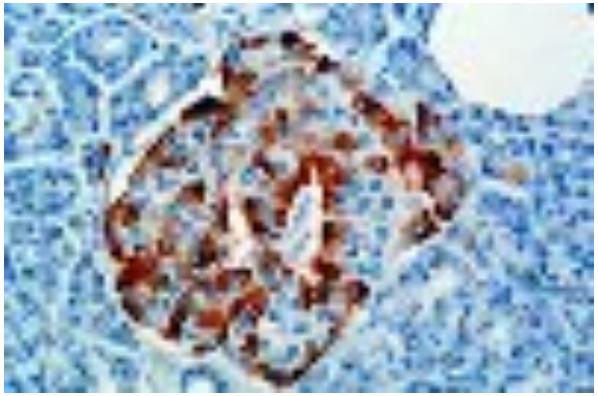
A leggyakoribb enzim-szubsztrát rendszerek

Peroxidáz (HRPO)	**DAB – diaminobenzidin (barna)**
	AEC – amino-ethyl-carbasol (vörös)
	True Blue (kék)
Alkalikus Foszfatáz (ALP)	**NBT – nitroblue tetrasolium (kék)**
	BCIP – bromo-chloro-indoyl foszfát (kék)

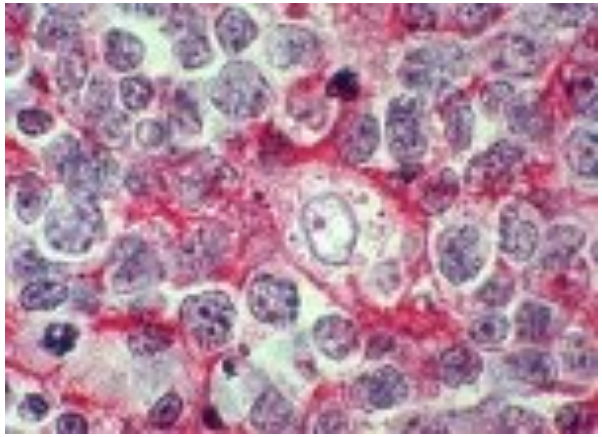
Immunhisztokémia



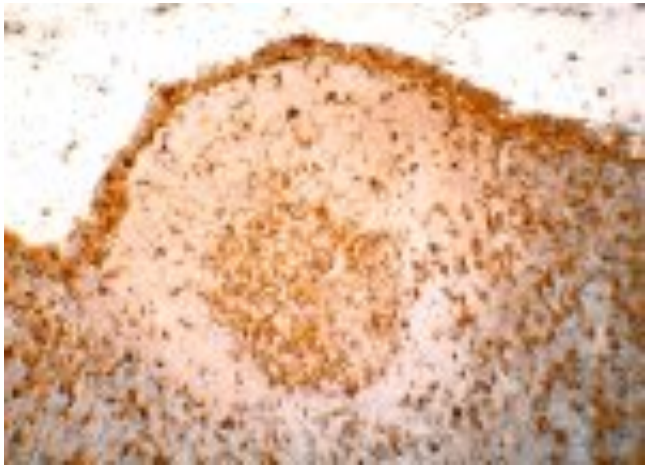
Pancreas, anti-insulin



Pancreas, anti-glukagon

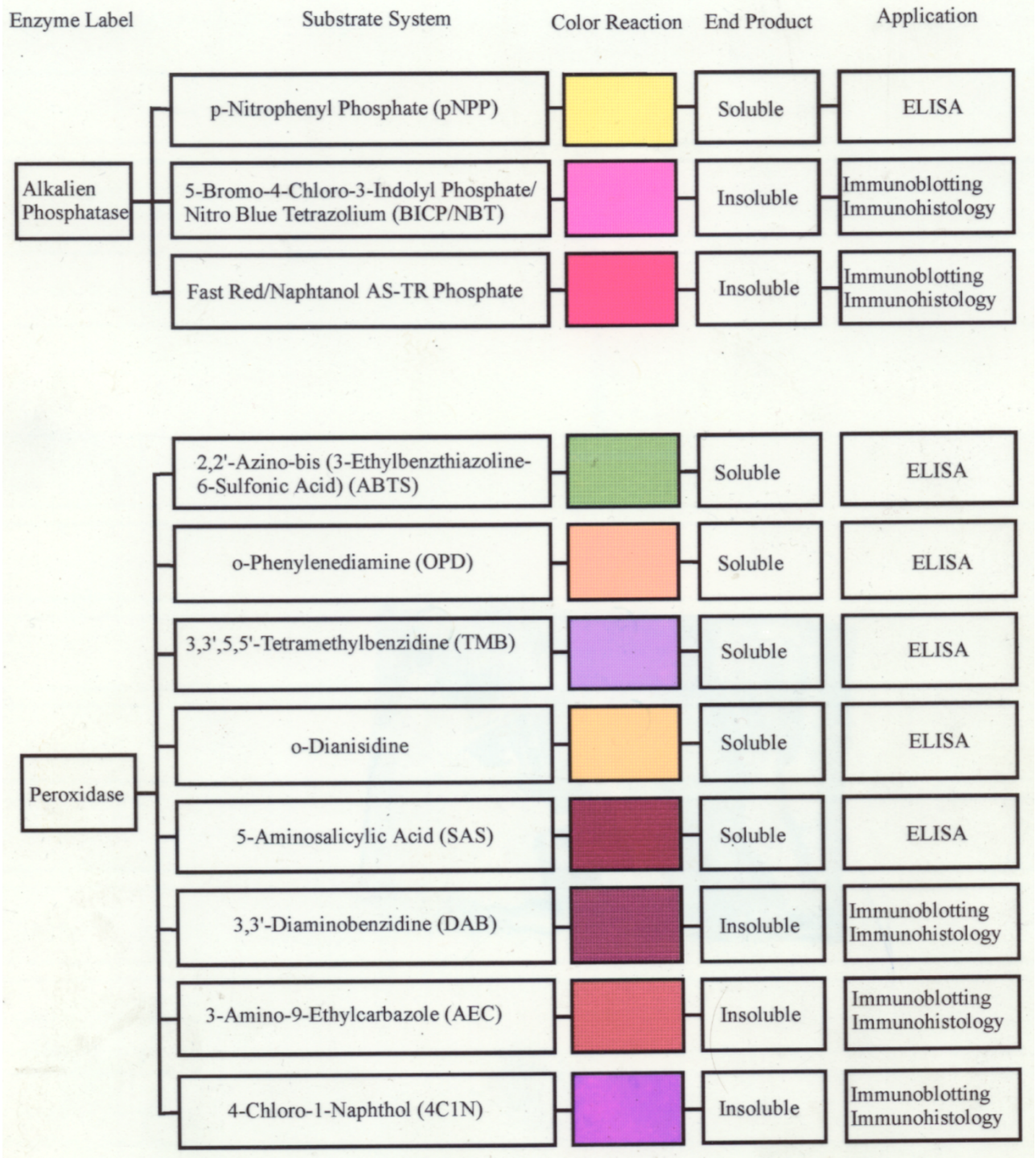


FDC anti-CD21



DC anti-p55 (nyirokcsomó)

Szubsztrát rendszerek

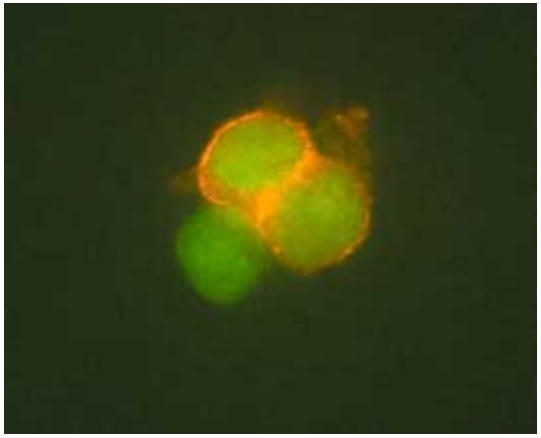


Fluorochrome Specifications

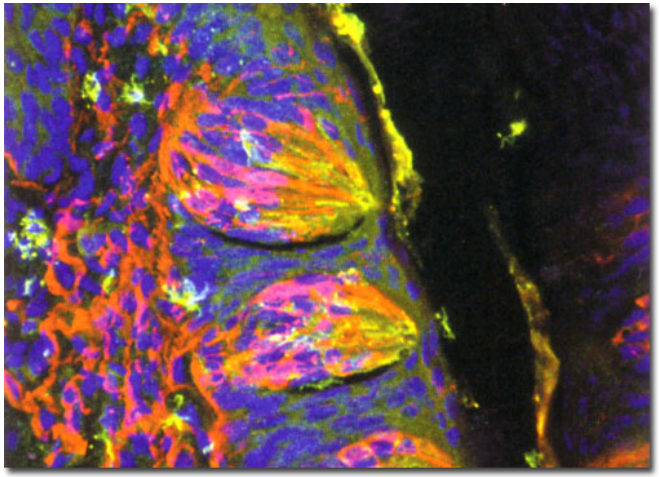
Fluorochrome	Fluoresence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)	Em-Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACStar Plus™	BD FACSVantage™ SE	BD™ LSR	BD™ LSR II	BD FACSAria™	BD FACSArray™
Alexa Fluor® 405	Blue	401	360, 405, 407	421				✓		✓	✓	
Pacific Blue®	Blue	410	360, 405, 407	455				✓		✓	✓	
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.*

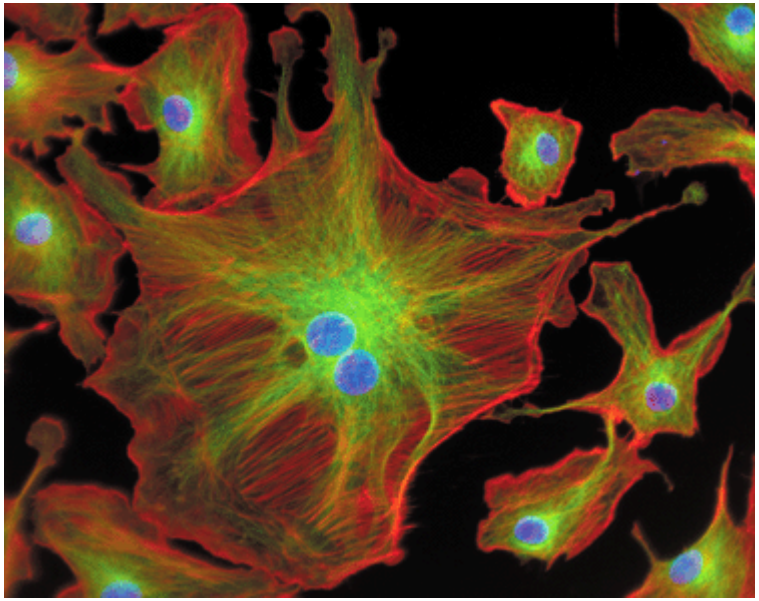
Immunfluoreszcencia



CD4+ T lymphociták



Patkány ízlelőbimbó



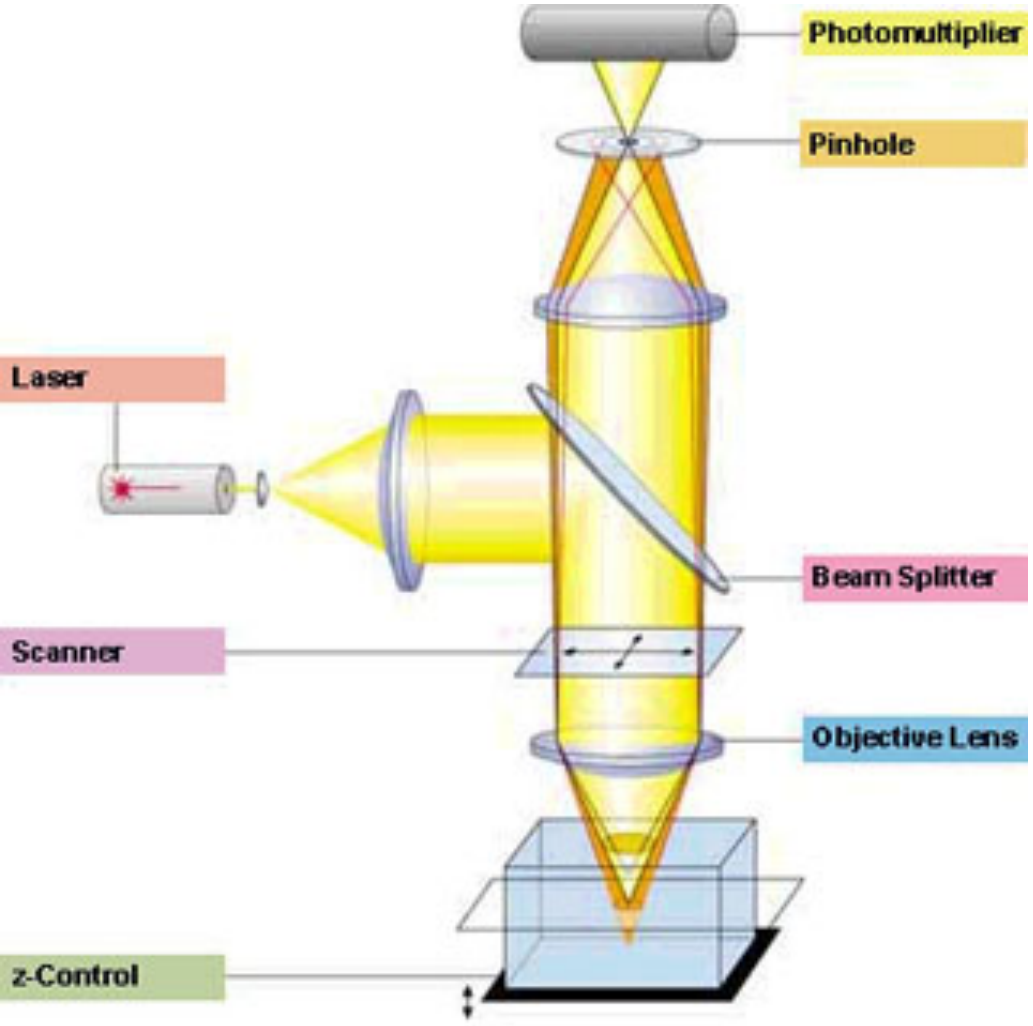
Endothel

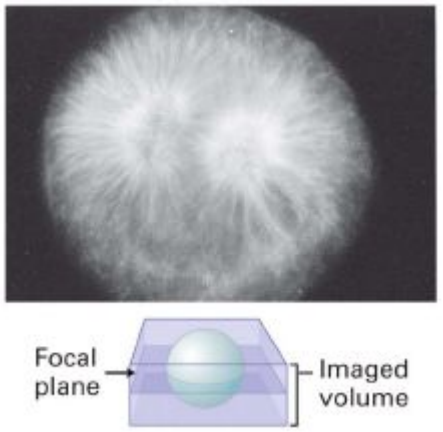
tubulin-zöld

actin-piros

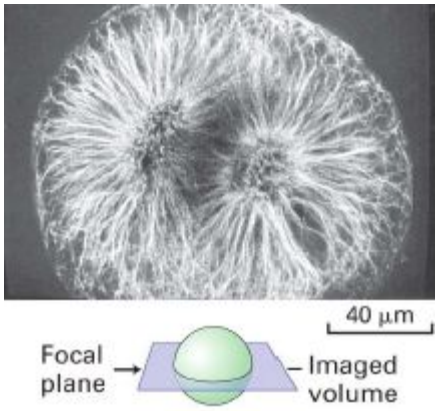
nucleus-kék

Konfokális mikroszkópia



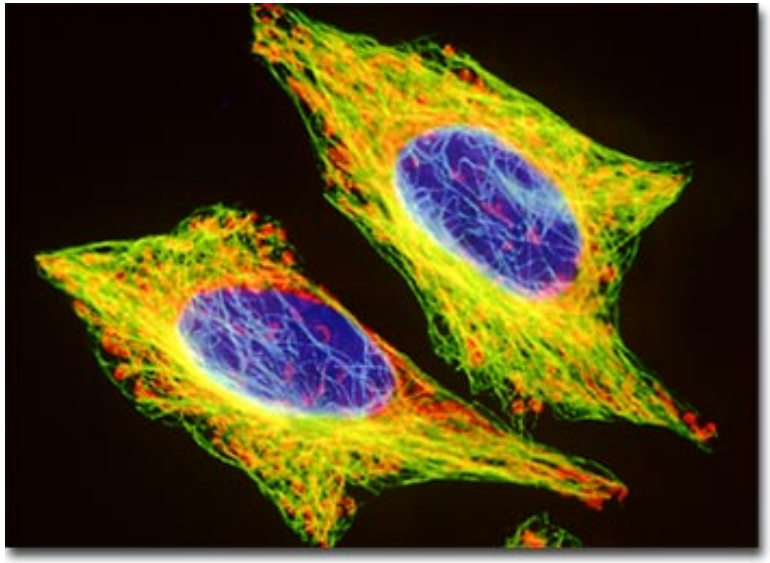


Konvencionális fluoreszcens mikroszkópos kép, anti-tubulin, mitotikus sejt

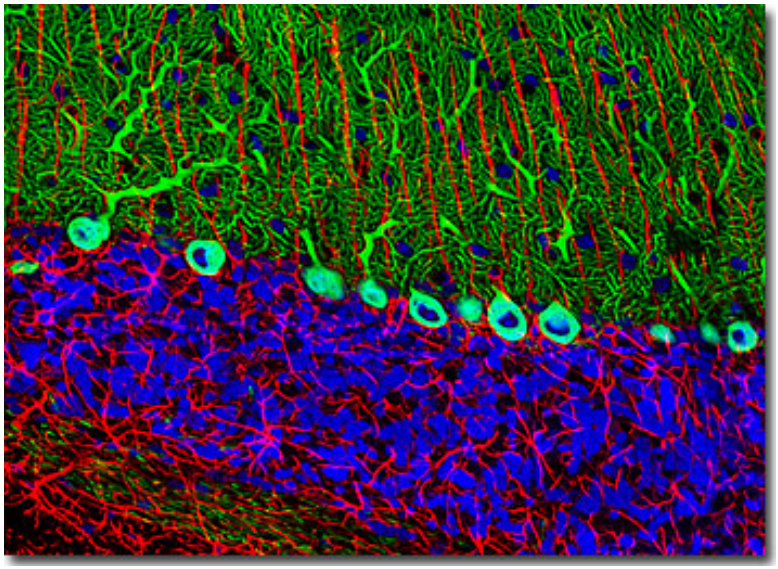


Konfokális mikroszkópos kép

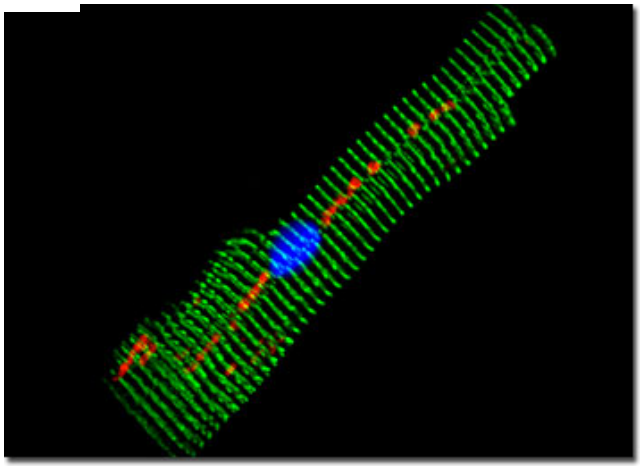
Konfokális mikroszkópia



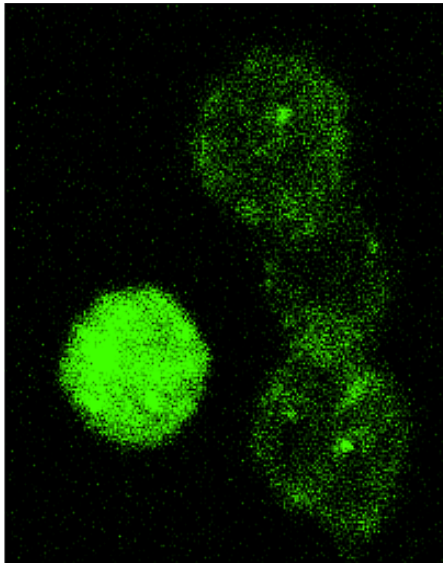
HeLa sejtek



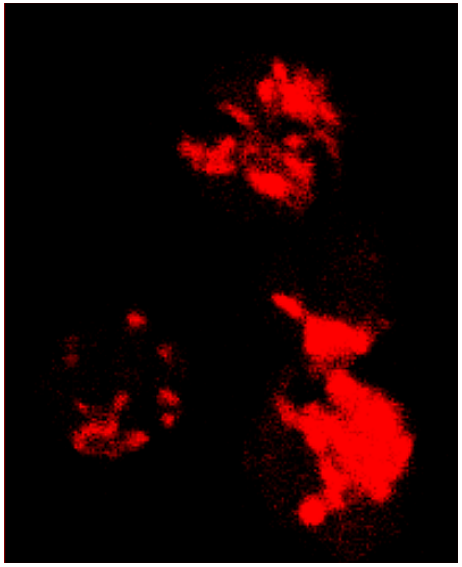
Patkány cerebellum



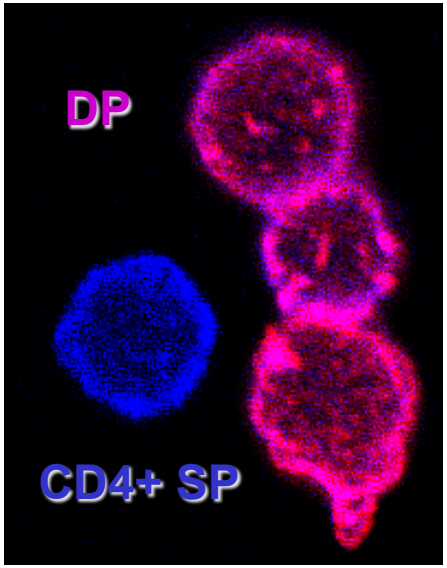
Szívizom



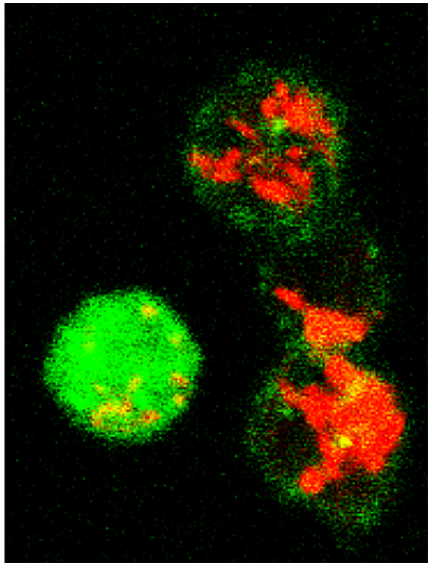
a-Glucocorticoid receptor-FITC



Chloromethyl-X-rosamine (Mitochondrium)



a-CD4-Pacific Blue
a-CD8-Alexa fluor 647



Chloromethyl-X-rosamine – GR merge

Lép – immunhisztokémia

Anti-egér Ig-HRPO (AEC)