



Sejtszámlálás másképpen: a Countess FL asztali berendezés alkalmazási lehetőségei

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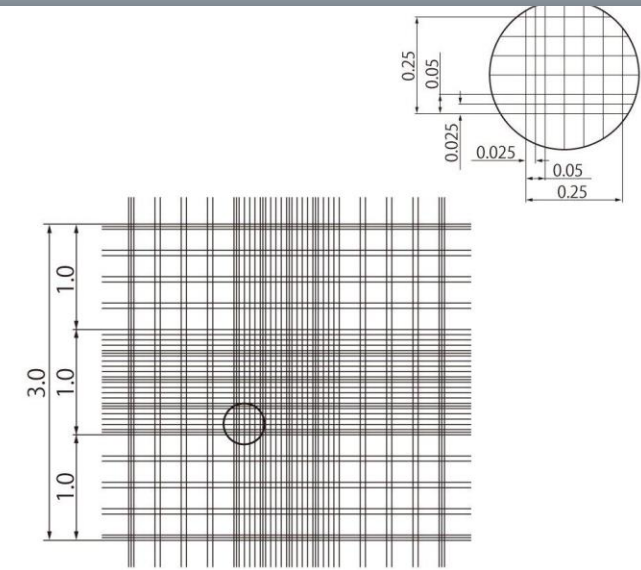
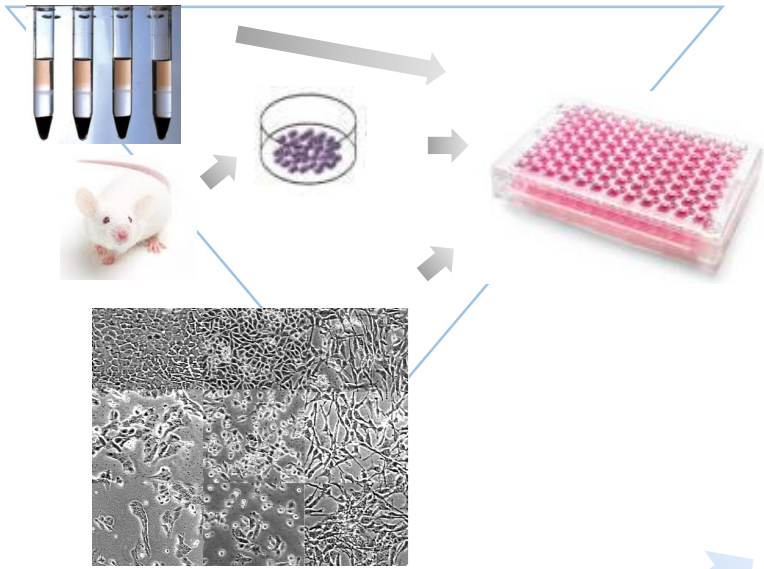
²Biomolekuláris Önrendeződés Kutatócsoport, MTA TTK

10. JUBILEUMI MedinProt konferencia
Budapest, 2018. november 10.

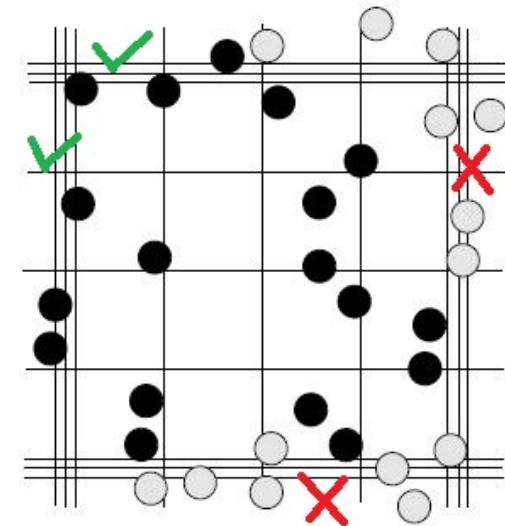
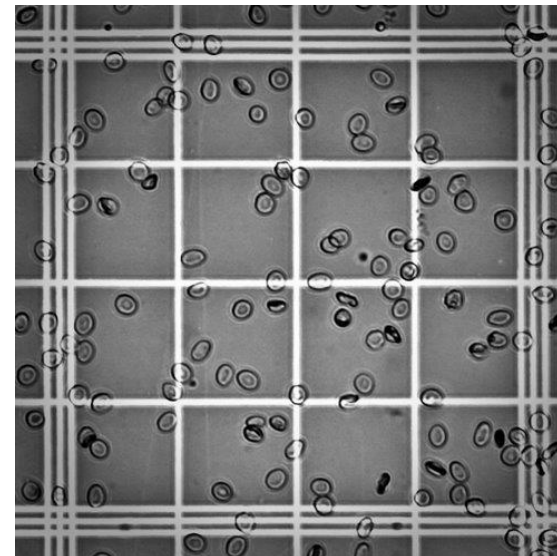
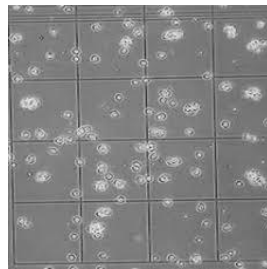


MEDINPROT

In vitro kísérletek, sejtszámlálás



1/10mmdeep, 1/400 & 1/25sqmm



INVITROGEN COUNTLESS II FL automata asztali sejtszámláló készülék



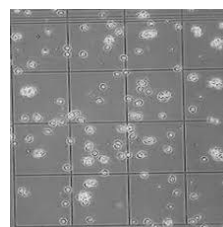
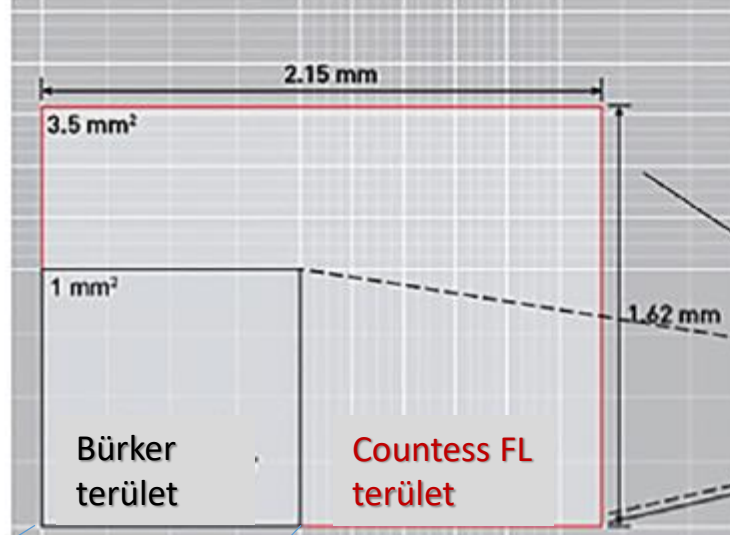
22,8 X 13,9 X 22,8 cm

3 csatorna

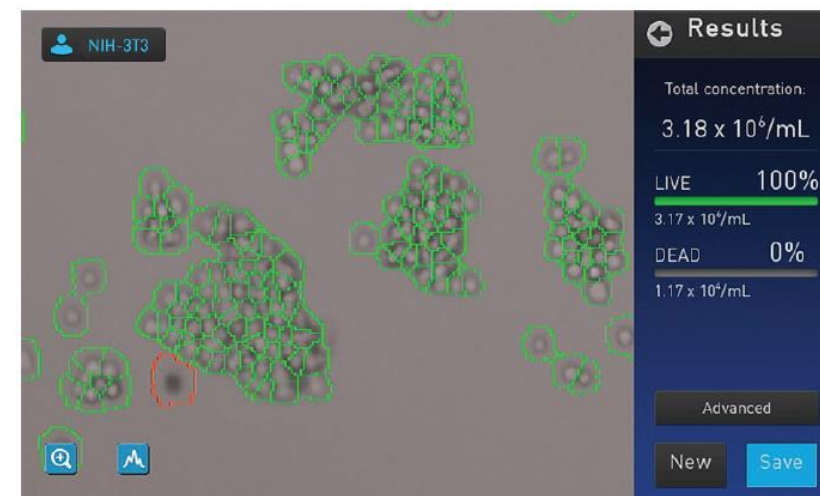
világos látótér (tripánkék)
2 fluoreszcens „light box”



Sejtek, elfoglalható terület: 2,15 x 1,62 mm



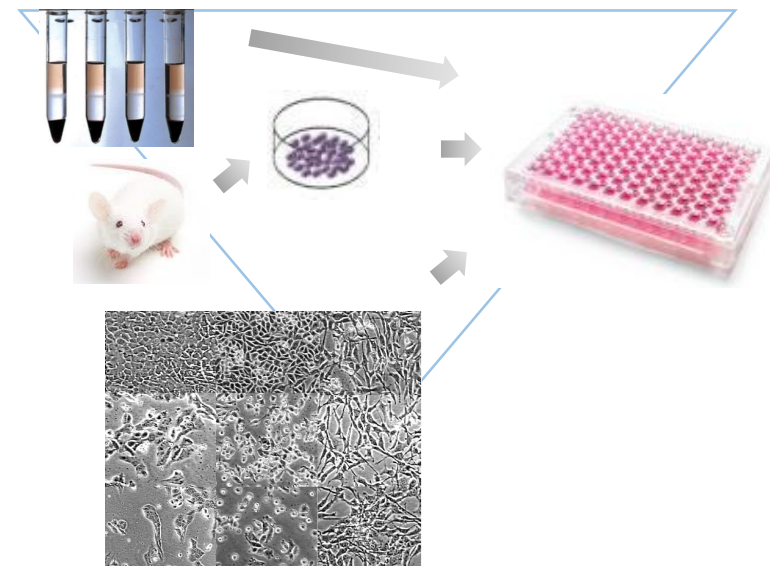
Sejtek mérete: 7-60 μm
Sejtszám tartomány: 1×10^4 - 1×10^7 /mL



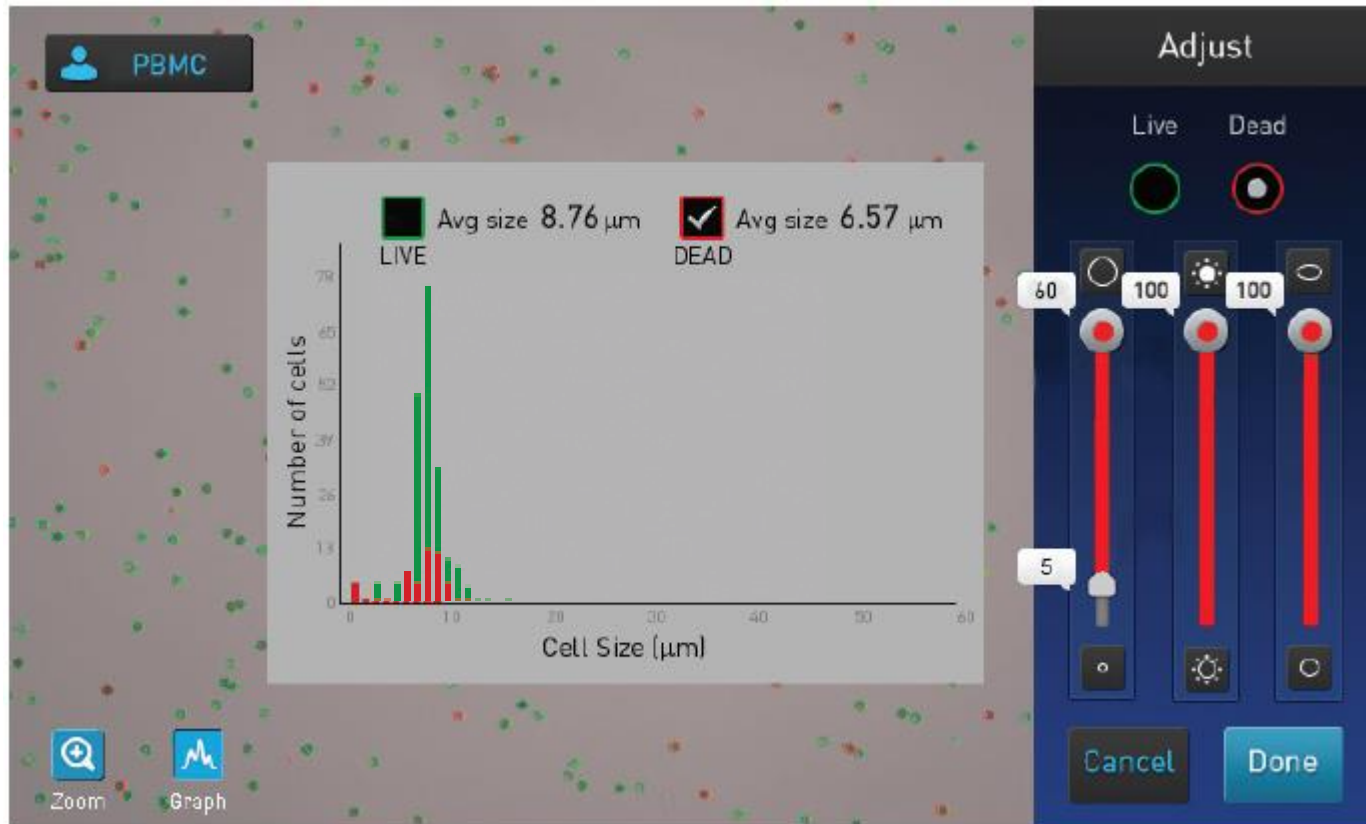
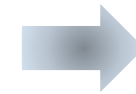
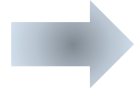
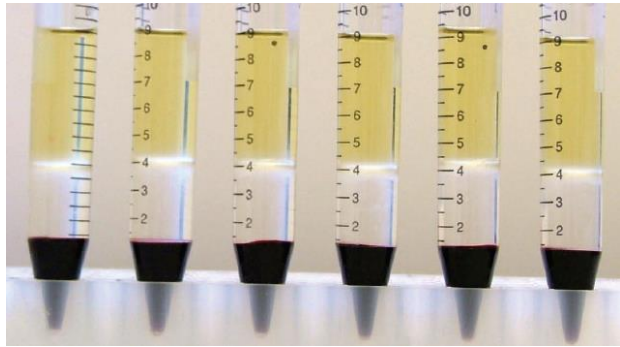
INVITROGEN COUNTLESS II FL: sejttípusok, validálás

Sejttípus	Faj	Szövet/Szerv	Sejtméret
A431	Human	Skin	15.5 μm
Adipocytes	Human	Adipose-derived stem cells	13 μm
Aortic smooth muscle	Human	Smooth muscle	20 μm
Blood, whole lysed	Human	Blood	NA
CHO-M1WT2	Chinese hamster	Ovary	NA
CHSE	Chinook salmon	Embryo	16–17 μm
COLO-205	Human	Colon	NA
COS-7	African monkey	Kidney	NA
HEK293	Human	Kidney	13 μm
HeLa	Human	Cervix	NA
HepG2	Human	Liver	18 μm
HL-60	Human	Blood	NA
J774A.1	Mouse	Blood	13–14 μm
Jurkat	Human	Blood	12 μm
K562	Human	Bone marrow	NA
MCF-7	Human	Breast	20–24 μm
MRC-5	Human	Lung	18 μm
NIH/3T3	Mouse	Embryo	18 μm
PBMC	Human	Blood	7–8 μm
PC-12	Rat	Adrenal gland	12–14 μm
Pulmonary artery endothelial cells	Human	Blood vessel	13 μm
Pulmonary artery smooth muscle	Human	Smooth muscle	20 μm
SF-21	Insect	Ovary	NA
U266	Human	Blood	12–13 μm
U2OS	Human	Bone	NA
Umbilical vein endothelial cells	Human	Blood vessel	17 μm

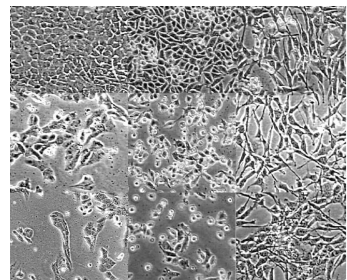
különböző primer
és folytonos (tumor eredetű) sejtkultúrákon



Perifériás vérből izolált mononukleáris sejtek életképességének vizsgálata



INVITROGEN COUNTLESS II FL: sejtszámlálás, hígítási adatok



Dilution Calculator

Current concentration: 5.63×10^5 /mL Live cells

What is your desired cell concentration? x Live cells/mL Total cells

How many mL do you need?

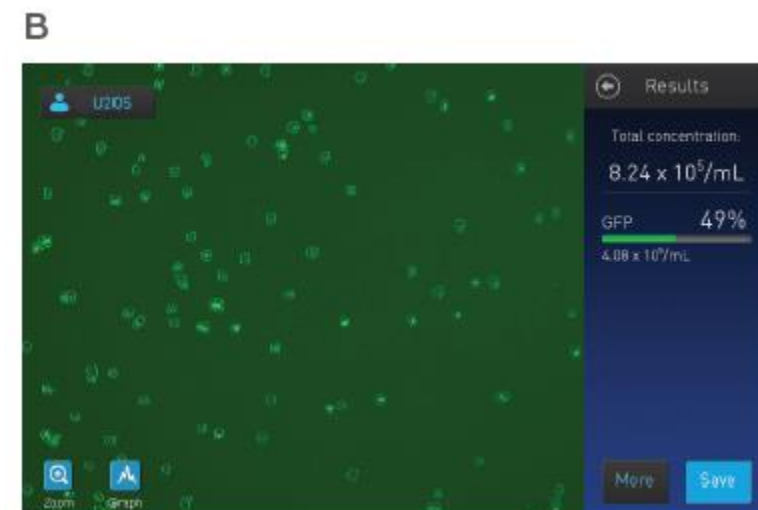
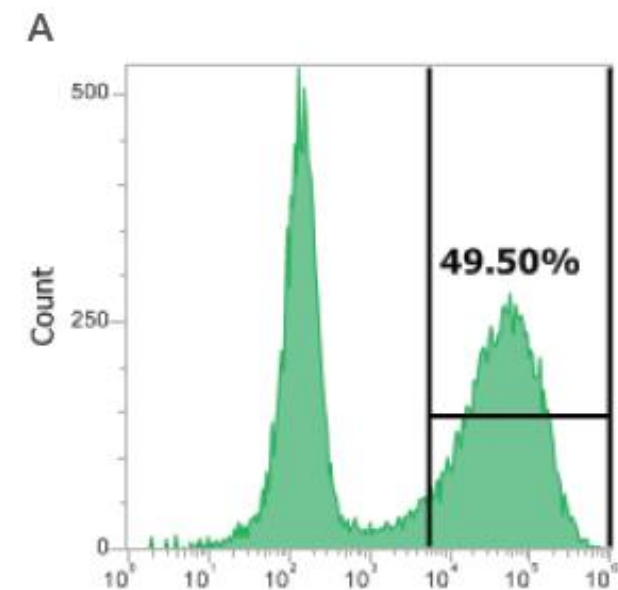
Mix **1.56** mL of your cell solution with **10.44** mL buffer.

INVITROGEN COUNTLESS II FL szűrők és azok specifikációja



Light cube	Excitation (nm)	Emission (nm)	Common compatible dyes/fluorescent proteins
DAPI	357/44	447/60	DAPI, Hoechst, BFP
CFP	445/45	510/42	ECFP, Lucifer Yellow
GFP	470/22	510/42	GFP, Alexa Fluor™ 488, SYBR™ Green, FITC
YFP	500/24	524/27	EYFP, acridine orange
RFP	531/40	593/40	RFP, Alexa Fluor™ 546, Alexa Fluor™ 555, Cy®3, DsRed, Rhodamine Red, dTomato
Texas Red™	585/29	624/40	Texas Red, Alexa Fluor™ 568, Alexa Fluor™ 594, MitoTracker™ Red, mCherry
Cy®5	628/40	692/40	Cy®5, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™

For a complete list of available common and specialty light cubes, go to [thermofisher.com/evoslightcubes](https://www.thermofisher.com/evoslightcubes)



INVITROGEN COUNTLESS II FL szűrők és azok specifikációja

invitrogen

Click the name of a light cube to view the list of compatible dyes

Commonly used light cubes

DAPI

TagBFP

CFP

GFP

YFP

RFP

Texas Red™

Cy5

EVOS Light Cube selection guide

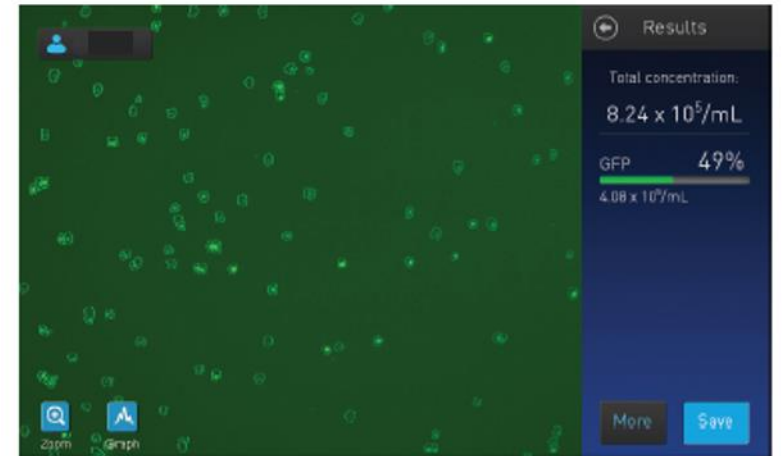
Invitrogen™ EVOS™ Light Cubes integrate LEDs and high-performance filters in a novel illumination system that is designed to deliver precise control, minimal maintenance, and exceptional reliability. These LED cubes are compatible with the EVOS FL and FL Auto systems as well as the Countess™ II FL Automated Cell Counter. Use the selection guide below to choose the light cube that best matches your dye.

Light cube	Dye	Abs peak	Em peak	Spectral match
GFP	1,8-ANS	372	480	Excellent
	4-methylumbelliferone	360	440	Excellent
	7-amino-4-methylcoumarin (AMC)	351	430	Excellent
	7-hydroxy-4-methylcoumarin	360	449	Excellent
	ABQ	344	445	Excellent
	Acridine	362	462	Excellent
	Alexa Fluor™ 350	346	442	Excellent
	Alexa Fluor 405	401	421	Fair

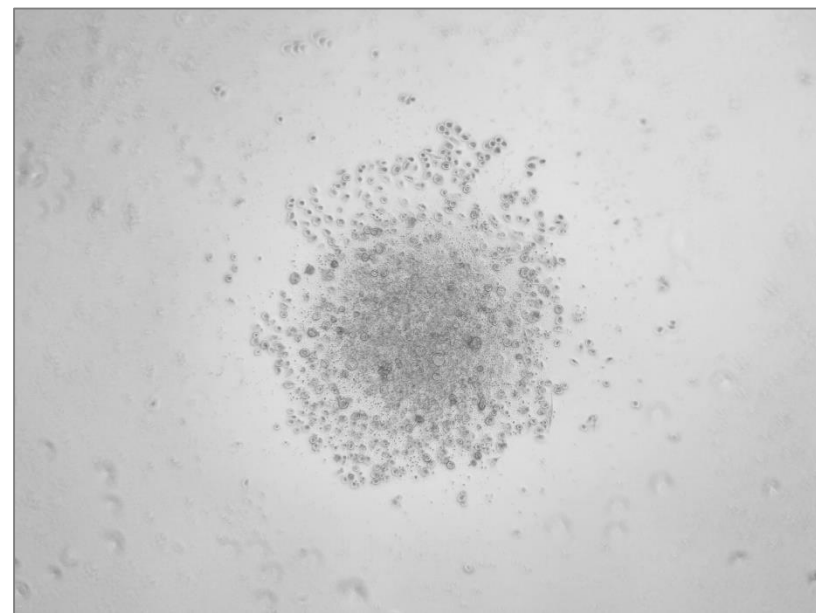
Light cube	Dye	Abs peak	Em peak	Spectral match
GFP (470/22 Ex; 510/42 Em) Cat. No. AMEP4651	5-Carboxyfluorescein (5-FAM)	492	518	Good
	Acridine orange + DNA	500	526	Good
	Acridine yellow	470	550	Excellent
	ActinGreen™ 488 (Alexa Fluor 488 phalloidin)	496	518	Excellent
	Alexa Fluor 430	432	540	Good
	Alexa Fluor 488	496	518	Excellent
	ATTO 465	453	507	Excellent
	Auramine	460	540	Excellent
	Aurophosphine	470	540	Excellent
	BCECF	499	540	Good
	BODIPY™ 492/515	490	540	Good
	BODIPY 505/515	502	540	Good
	BODIPY FL	502	540	Good
	BTC	464	540	Excellent
	Calcein	494	540	Good
	Calcein Green™ 1	506	540	Fair
		482	540	Excellent

Viability dye	Light cube	Cat. No.
ReadyProbes™ Cell Viability Imaging Kit, Blue/Green	DAPI; GFP	R37609
ReadyProbes™ Cell Viability Imaging Kit, Blue/Red	DAPI; RFP or Texas Red™	R37610
LIVE/DEAD™ Viability/Cytotoxicity Kit	GFP; RFP or Texas Red	L3224
Propidium Iodide ReadyProbes™ Reagent	RFP	R37108
SYTOX™ Green Nucleic Acid Stain	GFP	S7020
SYTOX™ Red Dead Cell Stain	Cy®5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy5	A1310

Balb/c egér lépsejtek proliferációjának vizsgálata, a kísérlet terminálási időpontja



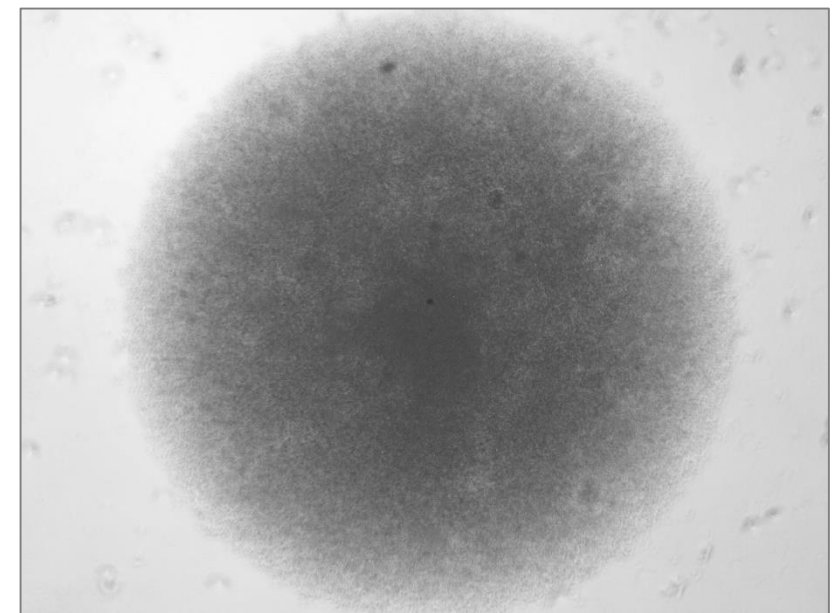
stimulálás, inkubációs periódus, időfüggés



Kontrol (5%)

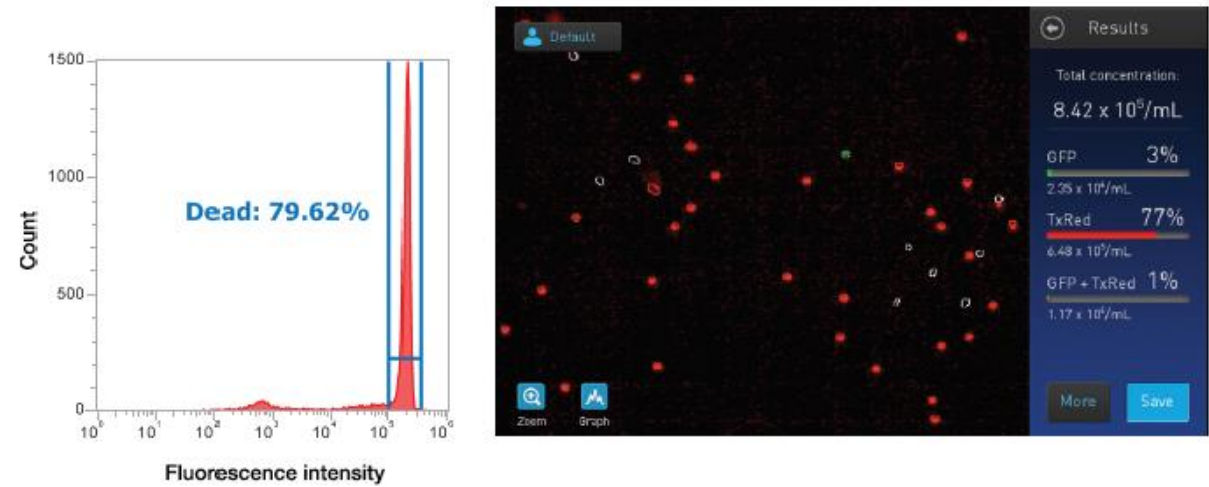
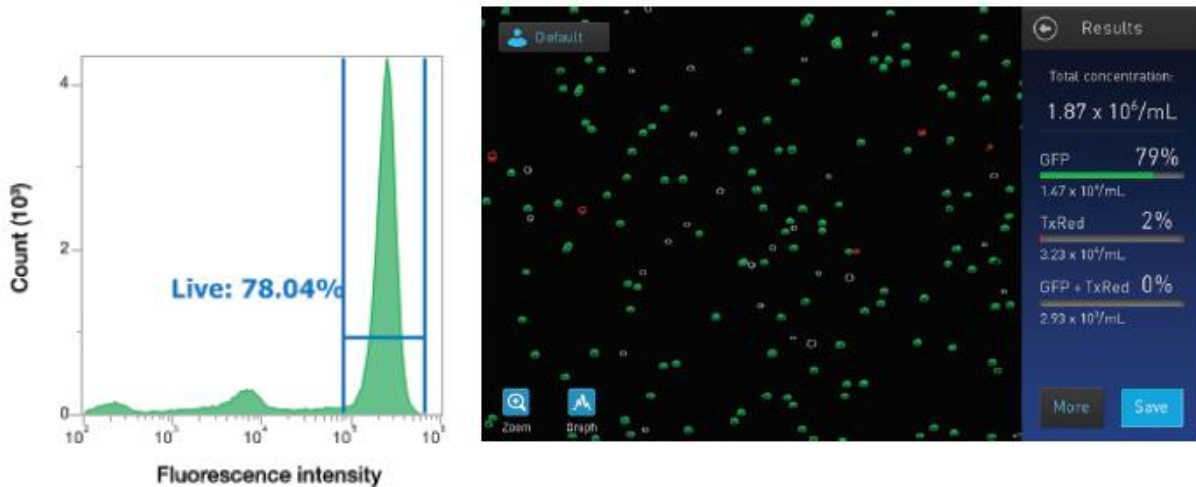
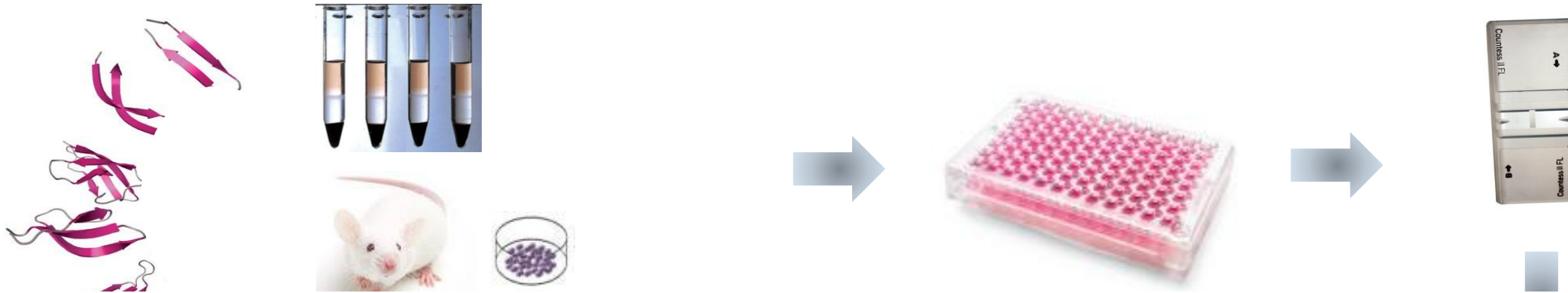


Peptid (49 %)



ConA (85%)

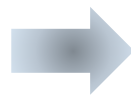
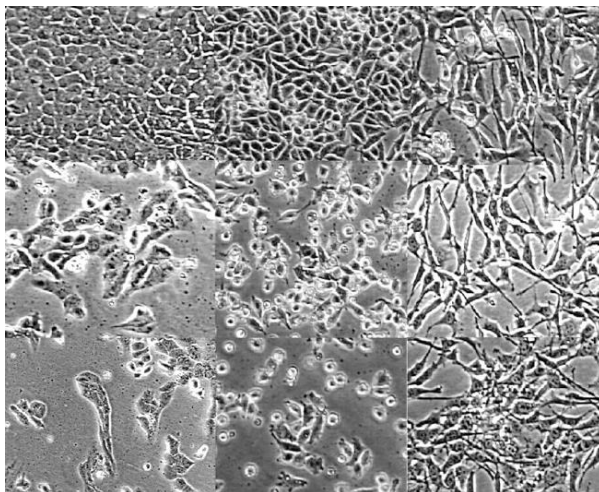
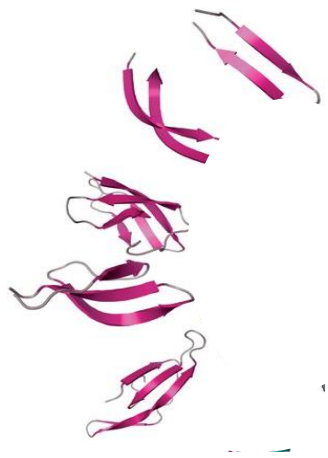
Citotoxicitás/életképesség meghatározása



Viability dye	Light cube	Cat. No.
ReadyProbes™ Cell Viability Imaging Kit, Blue/Green	DAPI; GFP	R37609
ReadyProbes™ Cell Viability Imaging Kit, Blue/Red	DAPI; RFP or Texas Red™	R37610
LIVE/DEAD™ Viability/Cytotoxicity Kit	GFP; RFP or Texas Red	L3224
Propidium Iodide ReadyProbes™ Reagent	RFP	R37108
SYTOX™ Green Nucleic Acid Stain	GFP	S7020
SYTOX™ Red Dead Cell Stain	Cy®5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy5	A1310



Apoptotikus események követése, detektálása



stimulálás, inkubációs periódus, idő- és koncentrációfüggés

Viability dye	Light cube	Cat. No.
ReadyProbes™ Cell Viability Imaging Kit, Blue/Green	DAPI; GFP	R37609
ReadyProbes™ Cell Viability Imaging Kit, Blue/Red	DAPI; RFP or Texas Red™	R37610
LIVE/DEAD™ Viability/Cytotoxicity Kit	GFP; RFP or Texas Red	L3224
Propidium Iodide ReadyProbes™ Reagent	RFP	R37108
SYTOX™ Green Nucleic Acid Stain	GFP	S7020
SYTOX™ Red Dead Cell Stain	Cy®5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy5	A1310



Köszönetnyilvánítás

Kapcsolódó pályázatok

OTKA 115431, 124077, 104275

Tématerületi kiválósági támogatás-Diagnosztikai
és Terápiás fejlesztések (20460-3/2018/FEKUTSRAT)

MTA Lendület Program (LP2016-2)

BIONANO_GINOP-2.3.2-15-2016-00017

NVKP_16-1-2016-0007

Versenyképes Közép-Magyarország Operatív Program
(VEKOP-2.3.2-16-2017-00014, VEKOP-2.3.3-15-2017-00020)

HunProtEx

Oktatás

Kémia, Biológia MSc hallgatók

PhD hallgatók

