BioResearch

Lonza

BITAB 毕特博生物 BITAB Biotech

Nucleofector™ Technology
4D转染系统全方位满足您的需求

Nucleofector™ Technology
for Hard-to-transfect Cells

Introduction: Nucleofector™ Technology

随着系统生物学与交义学科方法的应用，要求细胞和系统模型越来越接近体内细胞的功能。这就意味着将来的细胞转染多数会是原代细胞的转染。而传统的方法很难成功转染原代细胞。另外即使使用细胞系做为系统模型，传统的方法也难重复出好的细胞转染效率与成活率。Nucleofector技术就从根本上解决这个问题，无论是原代细胞、干细胞、还是细胞系，它每次都可重复出很高的转染效率。

1998年，Nucleofector技术开发成功，2001年上市，是市场上第一个有效的、非病毒介导的、用于原代细胞与难转细胞系的转染方法。现在Lonza还在继续不断改革创新，为研究者推出更多更好的新产品。

原理

Nucleofection™技术是利用电击在细胞模上穿个小孔。并且综合优化了各种特定细胞转染程序与转染液，使得转染物质（如DNA，RNA等）不仅可以进入细胞质，而且可直接通过核膜进入细胞核。这使得细胞的转染效率最高可达99%。

Nucleofector™ Technology – the Superior Non-viral Method

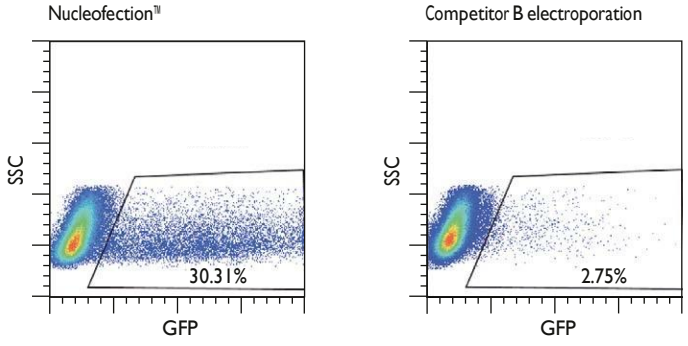


Figure 1. Transfection of the human natural killer cell line NKL using traditional electroporation and Nucleofection. 5 x 10^6 NKL cells were transfected with 2.5 µg of pmaxGFP™ Vector. Nucleofection: Nucleofector™ Solution V; Program O-017. Competitor B electroporation: 25 mV, 96 µF. Transfection efficiency was monitored by flow cytometry after 24 hours. Cells transfected by Nucleofection show a significantly better transfection efficiency compared to cells transfected by traditional electroporation. Cell viability, as measured 18 hours after transfection, was also superior using Nucleofection.
(Data courtesy of Dr. John Coligan, Laboratory of Immunogenetics, NIH/NIAID, Rockville, MD, USA. J Immunol Methods [2004] 284: 133-140.)

DNA Delivery Straight Into the Nucleus

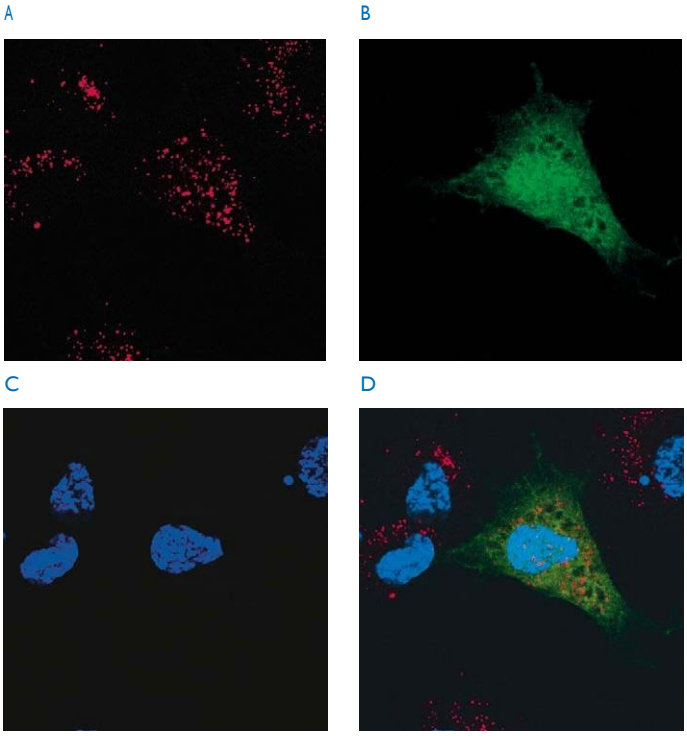


Figure 2. Normal human dermal fibroblasts (neonatal) were transfected with 2.5 µg TMR-labeled plasmid DNA encoding eGFP. After 2 hours, cells were fixed with 3.5% PFA and analyzed by confocal microscopy. TMR label is shown in (A), GFP fluorescence in (B), DAPI nuclear staining in (C) and a merge of all three fluorescent labels in (D).

Nucleofector™ 技术的组成

Nucleofector™技术由Nucleofector™仪器、细胞转染试剂和操作手册组成。

- Nucleofector™仪器， 内置针对每种细胞优化的电极参数， 参数可在仪器上直接选择也可用电脑软件选择。我们为大家提供了三种不同的平台。
- 试剂盒；试剂盒是包含有转染溶液，添加溶液，专用电极杯，吸管、pmaxGFP™阳性质粒。转染试剂为细胞转染提供保护，即保证了高的转染效率与成活率，又帮助细胞维持良好的生理功能性状。我们提供有一系列的优化转染试剂盒与操作手册。
- 数据库与操作手册；在我们的数据库中为大家提供600种以上细胞的转染数据和操作手册。优化的操作手册除了操作指导外，还包括细胞来源，传代，生长条件，培养基以及转染后培养等细节技巧。

Overview About Nucleofection Platforms

	Advanced Platform	96-well Add-on	High-throughput Platform	Basic Device
Device	4D-Nucleofector™ System	96-well Shuttle™ Device	HT Nucleofector™ System	Nucleofector™ 2b Device
Unit				
Throughput (samples per run)	Low to medium (1-16)	Low to high (1-96)	High (384)	Low (1)
Reaction volume	20 µL + 100 µL	20 µL	20 µL	100 µL
Electrode material	Conductive polymer	Conductive polymer	Conductive polymer	Aluminum
Low cell numbers (20 µL)	2 x 10^4 to 1 x 10^6	2 x 10^4 to 1 x 10^6	2 x 10^4 to 1 x 10^6	–
High cell numbers (100 µL)	2 x 10^5 to 2 x 10^7	–	–	2 x 10^5 to 2 x 10^7
DNA Vector amount/sample	0.2 – 1 µg (20 µL) 1 – 5 µg (100 µL)	0.2 – 1 µg	0.2 – 1 µg	1 – 5 µg
siRNA amount/sample (concentration 2nM–2µM)	0.2 – 200 pmol (100 µL) 0.04 – 40 pmol (20 µL)	0.04 – 40 pmol	0.04 – 40 pmol	0.2 – 200 pmol
Adherent Nucleofection (20 µL Nucleocuvette™ Strips)	■	■	–	–
Adherent Nucleofection (24-well culture plates)	■	–	–	–
Compatibility with 96-well Shuttle™ Device	■	–	–	–

最先进的平台

4D Nucleofector 系统提供更加灵活的多模块 New

基于大量客户的回馈，Lonza的工程师与科学家开发出的新一代创新性的4D-Nucleofector™转染系统，这个系统设计把兼容性最大化，使几种转染方式结合在一起，得到更好的转染效果，使用也更加方便。

4D-Nucleofector™系统是一种模块化系统，最基本的功能模块包括Core模块、X模块。各模块可以叠放在一起。您也可根据您的需求可以任意增减模块。

优点

操作简单灵活

- 不同的转染体系（20ul与100ul）使用相同的操作手册
- 100ul 转染细胞数量最多可达2 x 107
- 20ul 转染细胞数量最少到2 x 104

通量多元化

- 细胞通量的1-16样
- 可平行进行1-2 100ul 样品
- 可为100ul 和20ul样品预设50个以上参数
- 可根据您的要求选择20ul体系去减少试剂费用支出

转染原代细胞

- 5种试剂盒可覆盖所有原代细胞类型
- 转染优化实验更加简易便宜（如果您的细胞在数据库中没有需要自行优化）

保护细胞功能

- 贴壁转染模块（Y模块）无需消化就可转染，更好的保护细胞功能
- 4D系统用可转导电极，对细胞无金属离子毒害

1 The Core Unit –控制the 4D-Nucleofector™系统

- 运行软件去设计与保存实验
- 最多可联接5个功能模块（core, x, y, 96shuttle, CTU）
- 使用USB来升级软件系统，增加数据库

2 The X Unit –集合2个100ul 电极杯位置， 20ul 16个样的板条位置

- 可转染10^4到2X107的细胞

3 The Y Unit

- 无需消化，可直接在培养皿上进行转染

最具兼容性的模块： NucleofectorX模块

针对不同数量级的细胞，提供不同的电极杯。
X模可以使用两种不同形式的聚合材料电极，分别是如下电极杯与电极板条。

100ul 电极杯
新式可传导材料的电极杯替代了原来铝制电板杯
低通量但是一次可转大量的细胞
（适用于生化应用或 Western Blots）

可在相同的条件下转不同数量细胞

X模块使用的相同材质制成的20ul电极板条与100ul电极杯，X模块中不同的电极模式可选用相同的电转程序（系统可自动转化应用于不同模块的参数，客户无需进行额外操作）， 实现实验的方便性与灵活性最大化。

System, 96-well Shuttle™ Device and HT Nucleofector System).

一旦其中一种电极形式优化好，另一种就很容易转化好。不同通量的电转条件也很容易转化.[4D-Nucleofector™

Transferability Between Nucleofection Conditions Between Different Formats

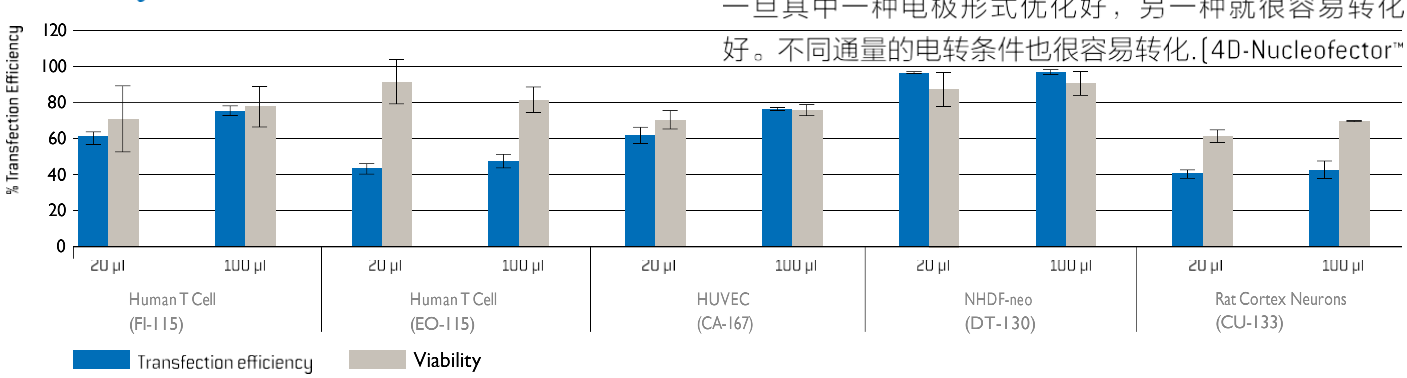


Figure 6. Various primary cells were transfected in the two Nucleocuvette™ vessel formats (20 µL and 100 µL) using the indicated programs. Twenty-four hours post Nucleofection cells were analyzed for transfection efficiency (flow cytometry) and viability (cell number normalized to no program control).

贴壁转染模块 4D-Nucleofector Y unit

至今为止基于电转的方法都是要求细胞处于悬浮状态Nucleofector™进入一个新时代，细胞可以在贴壁状态下直接转染。能够维持贴壁生长的细胞生理状态并完成转染。细胞培养在24孔板上，插入电极板即可完成转染。

优点

- 转染前与转染后都可在24孔板中
- 可在细胞生长的任何时期进行转染
- 成活率高，转染效率最高可达70%
- 与Clonetics原代动物神经元兼容

耗材

根据简化试剂盒的原则, 我们提供两种转染试剂AD1与AD2, 适用于所有细胞类型

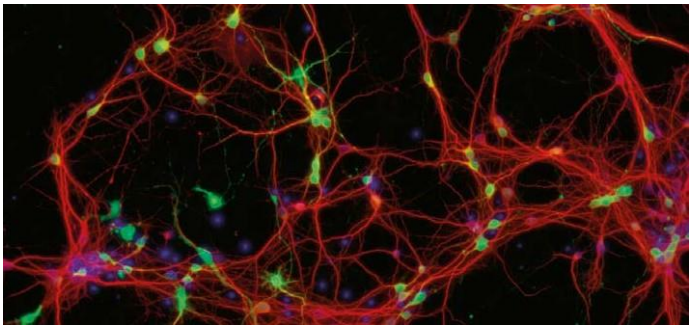


Figure 7. Efficient adherent Nucleofection of neurons in 24-well culture plates. Mouse cortical neurons were seeded into poly-D-lysine coated 24-well plates (1 x 10^5 cells/well). After 6 DIV, cells were transfected with pmaxGFP™ Vector using the AD1 4D-Nucleofector™ Y Kit. One day post Nucleofection, cells were stained by MAP2 antibody (red) and analyzed by fluorescence microscopy for maxGFP™ protein expression.

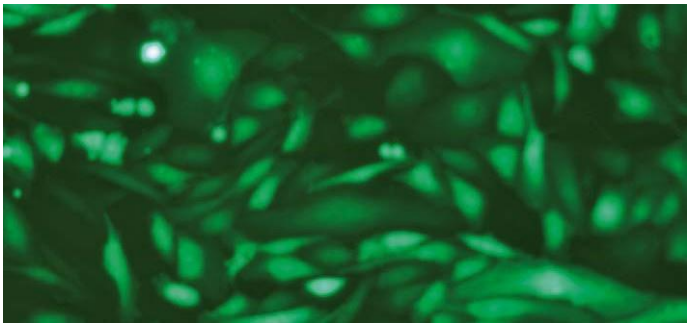
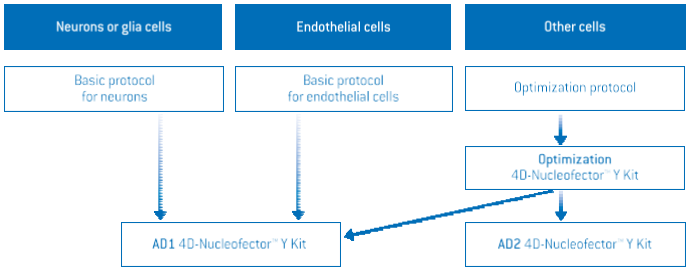


Figure 8. Human umbilical vein endothelial cells (HUVEC) were isolated and plated in passage 1 into collagen-coated 24-well plates at a density of 50,000 cells/well. After 1DIV cells were transfected with 16 µg pmaxGFP™ Vector using AD1 4D-Nucleofector™ Y Solution and program CA-215. Cells were analyzed for maxGFP™ Protein expression after 24h. (Data kindly provided by M. Sauvage, Pharmaceutical Industry, FR)



www.lonza.com/adherent-nucleofection

基本款: Nucleofector™ 2b Device

Nucleofector™2001年推出，用于实验室研究的单管系统，低通量有效转染不同底物到原代细胞与难转细胞系中。

Consumables

- 提供50多种原代细胞，血液细胞及干细胞试剂盒
- 5种细胞系试剂盒含概了所有种类细胞系
- CGMP试剂盒适用于蛋白生产应用

Benefits

高效的单管模式

- 转染DNA最高可达90%的转染效率
- 转染RNA最高可达99%的转染效率
- 同样也适用于多肽，蛋白质和小分子的转染

即方便又可重复实验结果

- 150多种针对不同细胞类型优化好的操作手册
- 细胞数据库包含有操作手岫以及650种细胞转染数据
- 转染成活率高，细胞功能完成，是可靠的可重复结果的保证
- 4000篇以上的文献

我们根据客户的需要提供大中小不同的包装，避免试剂的浪费

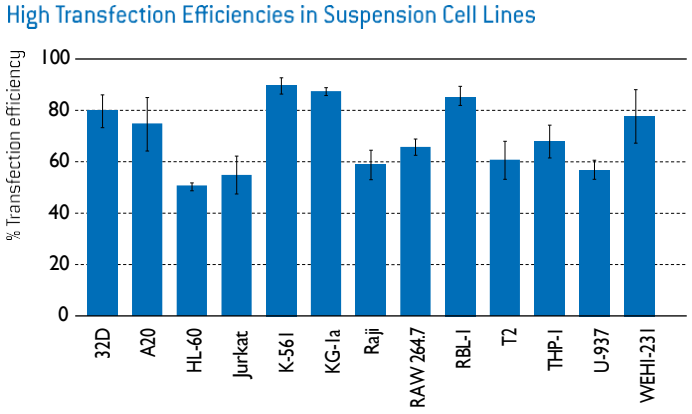


Figure 11. Transfection efficiencies 24 hours post Nucleofection in selected cell lines relevant for immunology research. Cells were transfected with either eGFP, maxGFP™ Reporter Protein or H-2KK and analyzed 24 hours post Nucleofection. Viability ranged from 60 – 90%.

Ordering Information

Description	Cat. No.	
Nucleofector™ Devices		
4D-Nucleofector™ Core Unit	AAF-1001B	–
4D-Nucleofector™ X Unit	AAF-1001X	Requires core unit to build complete system
4D-Nucleofector™ Y Unit	AAF-1001Y	Requires core unit to build complete system
4D-Nucleofector™ Guarantee, 2-year extension	AWE-1002	Has to be purchased at the time the device is purchased
4D-Nucleofector™ Service Contract	AWC-1001	Can be purchased at any time during the guarantee period
96-well Shuttle™ add-on (including laptop)	AAM-1001S	Requires core and X unit to build complete system
96-well Shuttle™ Guarantee, 2-year extension	AWM-1002	Has to be purchased at the time the device is purchased
96-well Shuttle™ Service Contract	AWB-1001	Can be purchased at any time during the guarantee period
HT Nucleofector™ System	AAU-1001	–
HT Nucleofector™ Installation and Training	AWT-1001	
HT Nucleofector™ Service Contract	AWU-1001	Can be purchased at any time during the guarantee period
Nucleofector™ 2b Device	AAB-1001	–
Nucleofector™ 2b Guarantee, 2-year extension	AWD-2002	Has to be purchased at the time the device is purchased
Nucleofector™ 2b Service Contract	AWA-2001	Can be purchased at any time during the guarantee period

	100 µL Nucleocuvette™		20 µL Nucleocuvette™; 16-well	Dipping Electrode
	12 rxn	24 rxn	32 rxn	24 rxn
4D-Nucleofector™ Kits				
P1 Primary Cell 4D-Nucleofector™ X Kit	V4XP-1012	V4XP-1024	V4XP-1032	–
P2 Primary Cell 4D-Nucleofector™ X Kit	V4XP-2012	V4XP-2024	V4XP-2032	–
P3 Primary Cell 4D-Nucleofector™ X Kit	V4XP-3012	V4XP-3024	V4XP-3032	–
P4 Primary Cell 4D-Nucleofector™ X Kit	V4XP-4012	V4XP-4024	V4XP-4032	–
P5 Primary Cell 4D-Nucleofector™ X Kit	V4XP-5012	V4XP-5024	V4XP-5032	–
Primary Cell Optimization 4D-Nucleofector™ X Kit	–	–	V4XP-9096 (96 rxn)	–
Basic Neuron 4D-Nucleofector™ X AD Kit (adherent)	–	–	V4XP-1A32	–
AD1 4D-Nucleofector™ Y Kit (adherent)	–	–	–	V4YP-1A24
AD2 4D-Nucleofector™ Y Kit (adherent)	–	–	–	V4YP-2A24
Optimization 4D-Nucleofector™ Y Kit (adherent)	–	–	–	V4YP-9A48
SE Cell line 4D-Nucleofector™ X Kit	V4XC-1012	V4XC-1024	V4XC-1032	–
SF Cell line 4D-Nucleofector™ X Kit	V4XC-2012	V4XC-2024	V4XC-2032	–
SG Cell line 4D-Nucleofector™ X Kit	V4XC-3012	V4XC-3024	V4XC-3032	–
Cell Line Optimization 4D-Nucleofector™ X Kit	–	–	V4XC-9096 (64 rxn)	–