For research use

HVJ Envelope transfection KIT

GenomONE™-Neo EX

siRNA data sheet

ISK ISHIHARA SANGYO KAISHA, LTD.

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Inspiration for Life Science

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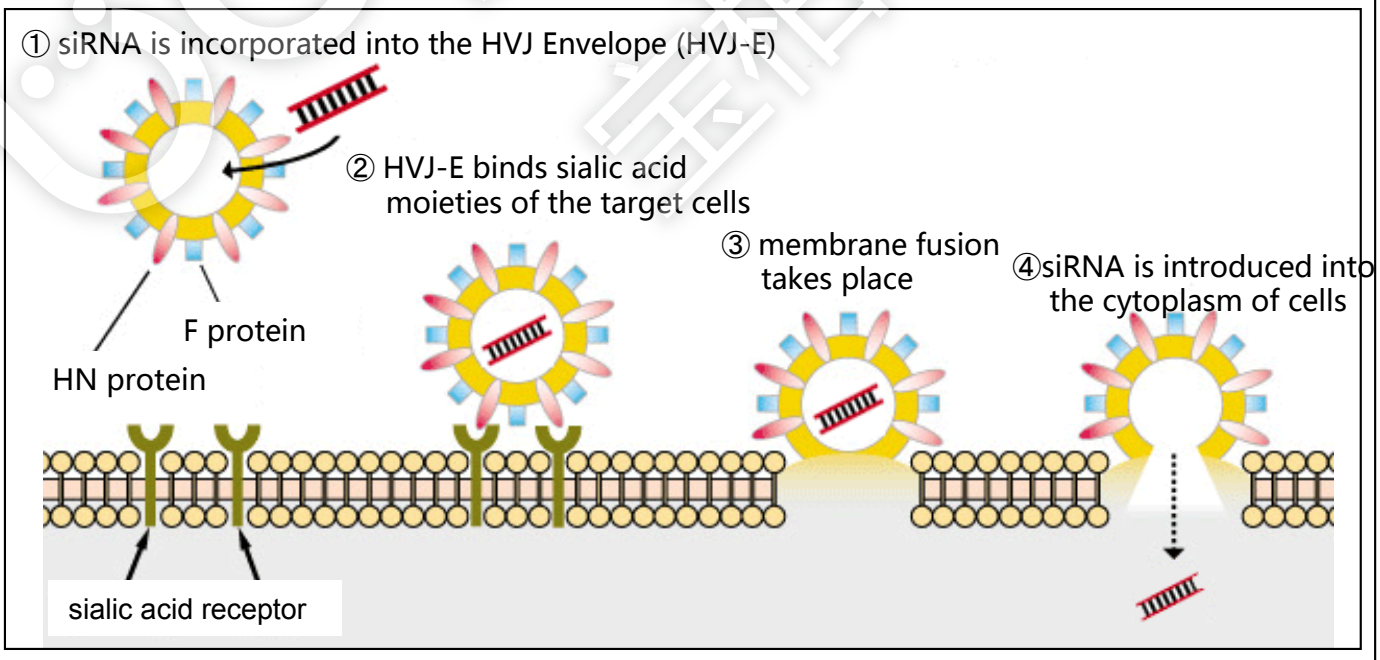
- **即使siRNA已经转入细胞，但Knock-down 效率太低，以至于无法做功能分析**
- **细胞毒性太大，无法准确评估**
- **需要可用于体内研究的siRNA转染试剂**

如果您有以上需求，请尝试使用
GenomONE™-Neo

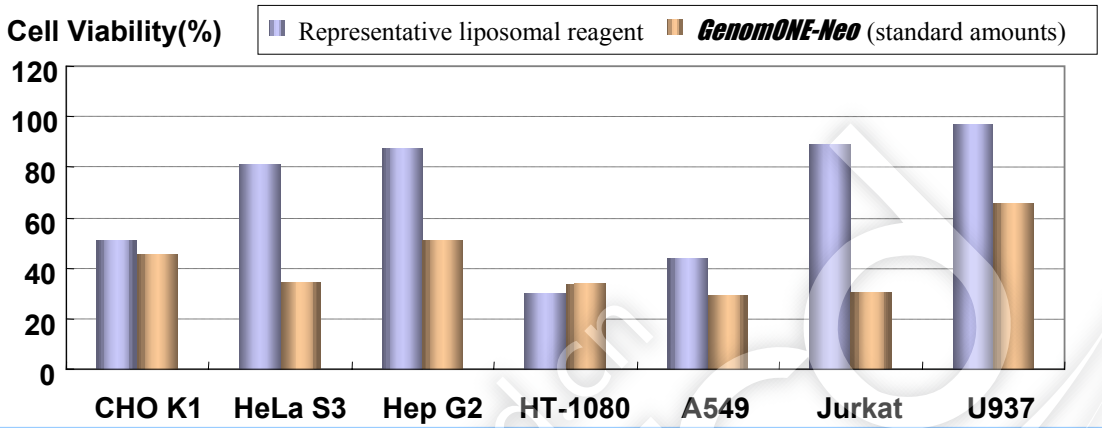
- **完全创新的方法，将siRNA直接导入细胞质**
  - 该系列产品通过仙台病毒（HVJ）包膜蛋白的膜融合能力，能够将siRNA有效的导入细胞。
- **可用于不同类型的细胞，包括贴壁细胞和非贴壁细胞，体外细胞研究和体内研究（作用于实验动物）**
- **可对不同类型样品进行快速siRNA转染**
  - 可用于高通量RNAi文库筛选。
  - 一个试剂盒（GN004EX）能够转16块24孔板（约400个孔）（使用标准siRNA操作）



Principle of siRNA transfer with ***GenomONE™-Neo***

GenomONE™ 和脂质体试剂导入siRNA效率比较

【案例1】Eg5 knock dowm抑制细胞增殖的效率

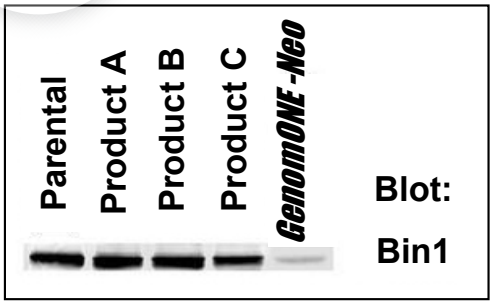


Eg5 knock-down会抑制细胞增殖、诱导细胞凋亡。
在96孔板中，每种试剂加入Eg5-siRNA的最终浓度为50 nM。用试剂处理以上所有细胞，48小时后，用WST-1测定细胞存活率。
对比发现，**GenomONE-Neo**对Eg5 knock-down的效率更强。

【案例2】 Lamin A/C siRNA转染（人脐静脉血管内皮细胞HUVEC）

将Lamin A/C靶向siRNA和对照siRNA（每种最终浓度均为50nM）分别导入HUVEC，48小时后通过Western blot测定。
使用**GenomONE-Neo**时，Lamin A/C蛋白的特异性击倒指出（道①）。在另一方面，当代表现有的脂质体试剂被雇用，核纤层蛋白A / C的表达的非特异性抑制注意到（车道④）。

【案例3】Bin-siRNA转染（小鼠成肌细胞C2C12）



※Parental：对照细胞没有用siRNA处理

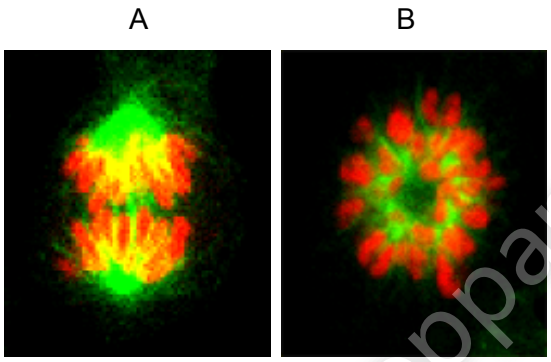
用传统转染试剂（A\B\C品牌）和**GenomONE-Neo**，将Bin-1 siRNA转入C2C12细胞，24小时后。

可观察到，**GenomONE-Neo**的knock-down效率明显高于其他产品。

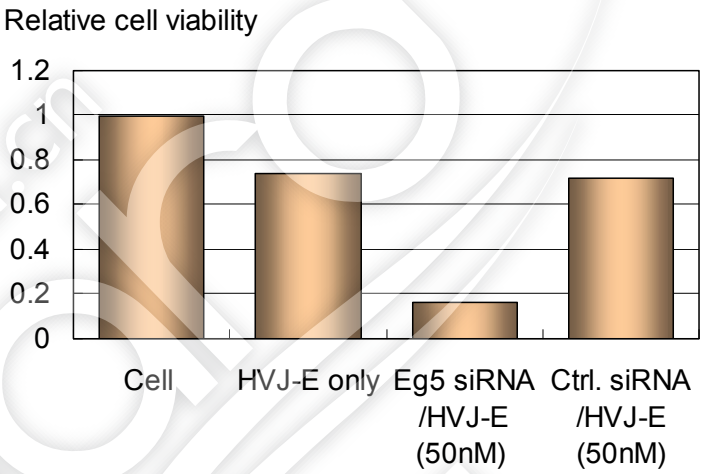
【数据来源】Dr. Chie Kojima* and Dr. Hisataka Sabe,
Department of Molecular Biology, Osaka Bioscience Institute, Japan.
* Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Japan.
【参考文献】C. Kojimaet al. EMBO Journal, 23, 4413-4422 (2004)

Knock-down驱动蛋白 Eg5 抑制HT800细胞增殖

① Knock-down Eg5 后，纺锤体的方向分布

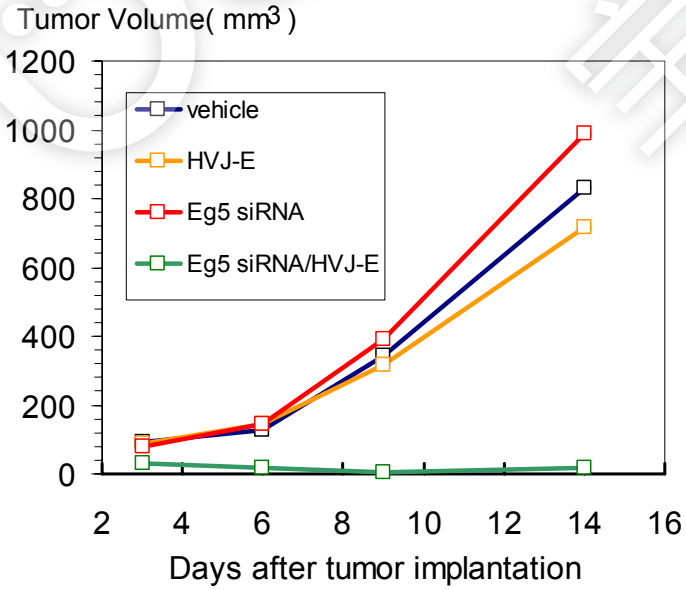


② Knock-down Eg5 诱导细胞凋亡



用**GenomONE-Neo** 将Eg5siRNA（50nM）转入HT-1080细胞。24小时后，在共聚焦激光扫描显微镜下观察细胞。对照细胞导入siRNA（①-A），在细胞分裂过程中，纺锤体（α微管蛋白；绿色）和染色体（DNA；红色）均正常。实验组细胞导入Eg5 siRNA，在细胞周期的中期，纺锤体和染色体的分离均出现异常（①-B），细胞分裂被抑制，从而导致细胞凋亡（②）。

③ Knock-down Eg5抑制小鼠皮下肿瘤生长（裸鼠皮下植入HT-1080细胞）

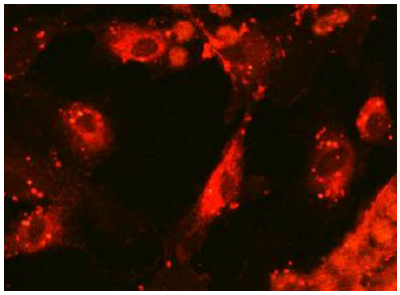


使用**GenomONE-Neo**在10 cm培养皿中将Eg5-靶标siRNA（56 nM）导入HT-1080细胞。孵育4小时后，收获细胞并皮下植入BA-LB/c小鼠（5×105细胞/小鼠）。在不同的时间点检测植入后的肿瘤体积，显示肿瘤几乎完全被抑制。而Eg5 siRNA单独处理组，肿瘤几乎没有被抑制。结果表明，HVJ-E可有效将siRNA导入细胞。

GenomONE-Neo

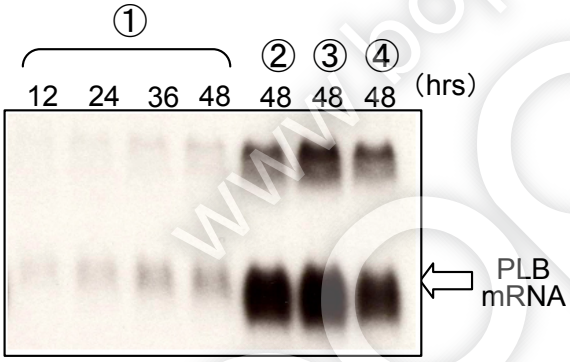
Knock-down 原代大鼠心肌细胞 受磷蛋白（PLB）

① 转染Cy3-标记 siRNA



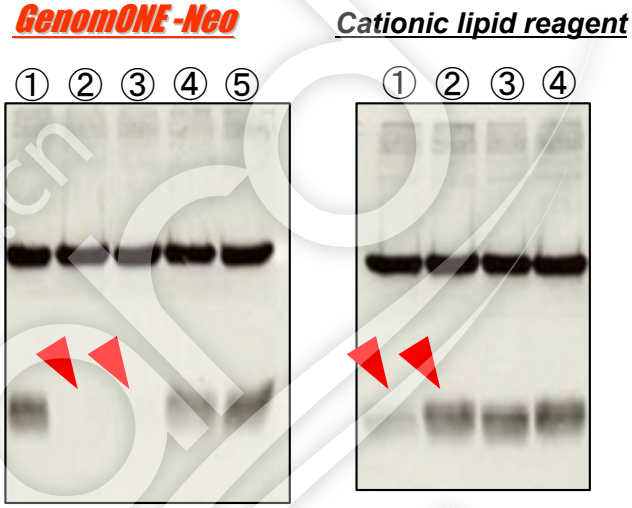
siRNA（30 nM）导入新生大鼠心肌细胞（原代培养）

② Northern blot检测



①:PLB siRNA（30nM）
②:Scramble siRNA（30nM）
③:HVJ-E only
④:Non-treated

③ Western blot 检测

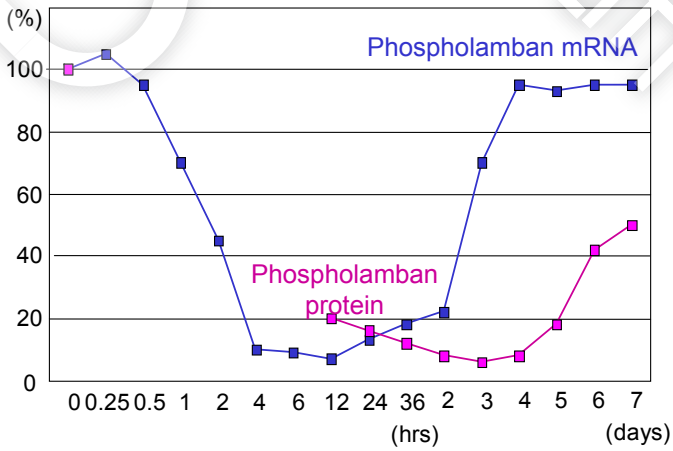


①:Non-treated
②:PLB siRNA (10 μg)
③:PLB siRNA (2 μg)
④:Scramble siRNA (10 μg)
⑤:HVJ-E only

①:PLB siRNA (10 μg)
②:PLB siRNA (2 μg)
③:Scramble siRNA (10 μg)
④:Non-treated

转染试剂对siRNA导入细胞的效率较高（80%-100%）。但是，与阳离子脂质体相比，**GenomONE-Neo**可以在较低siRNA浓度的情况下knock-down目标蛋白。

④ siRNA的长期持续活性



用**GenomONE-Neo**转染PLB靶标siRNA，转染效率达80%，在转染1~5天内，均有明显的PLB蛋白抑制效果。

【数据来源】Dr. Masashi Arai and Dr. Atai Watanabe；
Department of Medicine and Biological Science,Gunma University Graduate School of Medicine, Japan.

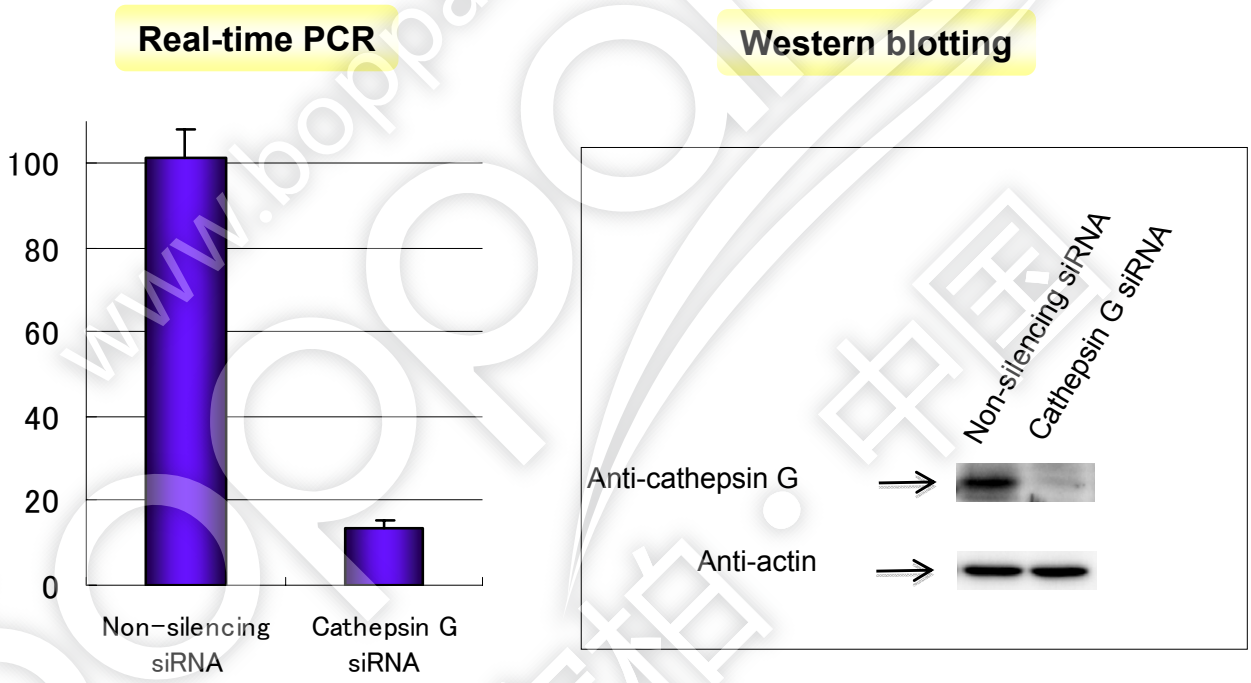
【参考文献】A. Watanabe et al. J. Mol. Cell. Cardiol., 37(3), 691-698 (2004)

GenomONE-Neo

难转染细胞 U937 RNAi

总所周知，用脂质体转染试剂和电转法，将siRNA转染U937人白血病单核细胞中非常困难，但是我们通过实验证明，利用**GenomONE-Neo**将siRNA转染细胞后，其knock-down的效率可达85%以上。

- siRNA转染后72小时收集细胞，分别用RT-PCR和Western blotting检测mRNA和蛋白量的变化。



siRNA转染后72小时收集细胞，分别用RT-PCR和Western blotting检测mRNA和蛋白量的变化。

转染Cathepsin G特异性siRNA后，其蛋白水平减少到15%

- 与脂质体或者电转法相比，**GenomONE-Neo**的转染效率更高，细胞毒性更低，因此knock-down效果更好。

【数据来源】Dr. Y. Tsuchiya (Department of Hygienic Chemistry, Showa Pharmaceutical University).
Present address: Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry.

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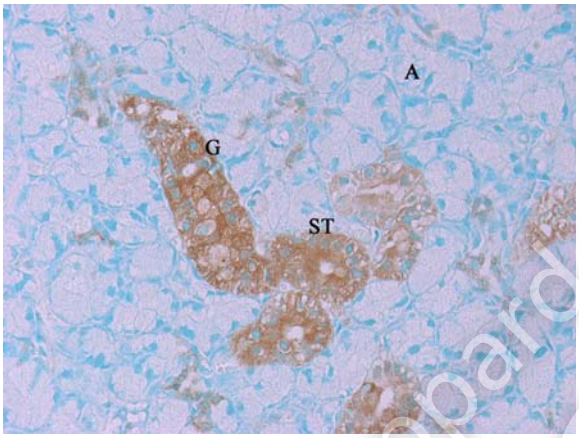
Y. Tsuchiya et al.: 4-Hydroxy-2-nonenal-modified glyceraldehyde-3-phosphate dehydrogenase is degraded by cathepsin G. Free Radical Biology & Medicine, 43, 1604-1615 (2007).

GenomONE-Neo

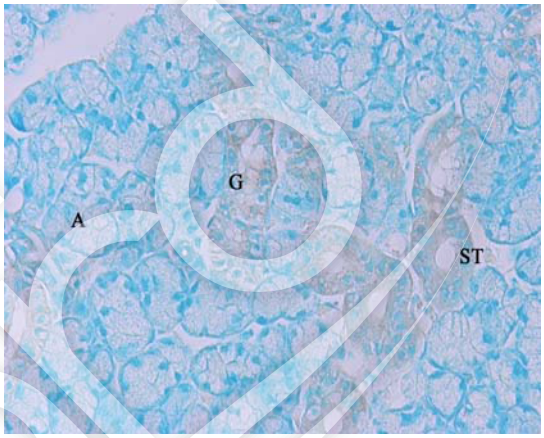
大鼠颌下腺逆行注射siRNA（体内）

Ca^{2+}-dependent Cl^- channel protein（rCLCA）表达的特异性抑制

免疫染色



non-injection



rCLCA siRNA-injection side

rCLCA siRNA（2 nmol）和**GenomONE-NEO**（2AU）混合后，以逆行注射的方式注入大鼠颌下腺。48小时后，用免疫染色检验siRNA注射对Cl-离子通道蛋白表达的抑制作用。

ST：纹状管，G：颗粒曲管，A：腺泡

ST:striated duct, G:granular convoluted tubule, A:acini

颌下腺中Cl^-重吸收抑制

	Treatment group	Electrolyte concentrations in final saliva（mM） Na^+	K^+	Cl^-	
(n=11)	rCLCA siRNA-injection sidea	17.5±1.3	35.3±1.2	**21.6±2.2**	★
	non-injection side (control)	16.7±0.9	34.6±1.3	**11.5±1.4**	★★
(n=9)	scrambled siRNA-injection side	17.7±1.3	33.8±1.4	**14.7±2.7**	
	non-injection side (control)	18.4±1.1	32.4±1.2	11.8±2.0	

Mean±SEM ★ p<0.001, ★★ p<0.05

rCLCA siRNA（2 nmol）混合**GenomONE-Neo**（2AU）逆行注射到大鼠颌下腺。48小时后，通过给药（pilocarpine毛果芸香碱）刺激唾液释放，收集分泌唾液。分析唾液中电解质的浓度，结果表明，注射rCLCA siRNA组Cl^-水平，明显高于对照组。证明，rCLCA siRNA注射后，可显著抑制Cl^-的重吸收，而对Na^+和K^+无影响。

注射siRNA，作用于囊性纤维跨膜电导调节（CFTR），造成Cl-重吸收的特异性抑制。

【数据来源】Dr. Kazunari Ishibashi
Department of Functional Bioscience, Fukuoka Dental College (Japan).

【参考文献】K. Ishibashi et al., J. Dent. Res., 85(12), 1101-1105 (2006).

Example of siRNA transfection

in vitro

Ref. No.	Cell	Origin	siRNA target
1	MIN6	Mouse pancreatic β cell	GPR40
2	Jurkat	Human acute T cell leukemia	SS-A/Ro52
3	C2C12	Mouse myoblast(differentiated)	Bin1
4	primary monocyte	Human monocyte	Caveolin-1
5	primary cardiac myocyte	Rat cardiac myocyte	phospholamban
6	U937	Human myelomonocytic cell	Brap2
7	K562	Human chronic myelogenous leukemia	Bim
8	HUVEC	Human umbilical vein endothelial cell	TSAd (T-cell specific adapter)
9	CMK6G3	Monkey ES cell (stably expresses EGFP)	EGFP
10	primary monocyte	Human monocyte	Tollip(Toll-interacting protein), IRAK-1(IL-1 receptor-associated serine/threonine kinase 1)
11	HuH-6, HuH-7, HepG2	Human hepatoblastoma cell, Human Hepatocecellular carcinoma cell	β-catenin
12	J774	Mouse macrophage cell	IL-13 Receptor α2
13	MIN6	Mouse pancreatic β cell	GPR40
14	primary T cell	Human peripheral blood	Human CARMA1
15	primary calvarial osteoblasts	Mouse calvarial osteoblasts	OPG (Osteoprotegrin)
16	primary mast cell	Mouse bone marrow-derived	GATA-1,GATA-2
17	INS-1E, NIH-3T3	Rat β cell, Mouse embryonic fibroblast	Sox6
18	primary macrophage	C3H mouse peritoneal resident	Mcl-1
19	primary granulosa cell	Mouse granulosa cell	Snap25 (Synaptosomal associated protein 25)
20	primary granulosa cell	Rat granulosa cell	TACE/ADAM17
21	BASMC	Bovine aortic smooth muscle cell	Bovine TE(tropoelastin)
22	U937	Human leukemic monocyte cell	Human cathepsin G
23	A549, H1299,TE13, PCNA-1, MIAPaCa-2, Du-145, ME-180	Human lung carcinoma, Human esophageal carcinoma, Human pancreas carcinoma, Human prostate carcinoma, Human cervical carcinoma	Ku80
24	HMVEC-dLyNeo(LEC)	Human neonatal dermal lymphatic microvascular endothelial cell	TLR4
25	U251MG, D54MG	Human glioma(p53 mutated at codon 273), Human glioma(p53 wild type)	Survivin, p53
26	granulosa cell	Mouse granulosa cell	TLR2, TLR4
27	granulosa cell	Mouse granulosa cell	Mkp3
28	COLO201	Human colon cancer	FKBP51
29	alveolar type II epithelial cells	Mouse alveolar type II epithelial cells	Nedd4, Nedd4-2
30	primary alveolar type2 cell	mouse lung alveolar type2 cell	LPCAT1

Example of siRNA transfection

in vivo

Ref. No.	Host	Target organ / tissue	Delivery Route	siRNA target
1	SCID mouse	i.d transplanted tumor (HeLa)	Intratumoral injection	Rad51
2	Mouse	Lung	Intratracheal injection	IL-13 Receptor α2
3	Rat	Submandibular gland	retrograde ductal injection	rCLCA(Ca^{2+}-dependent cl^- channels), CFTR(cystic fibrosis transmembrane conductance regulator)
4	Mouse	Colon	intrarectall injection	IRF4
5	Rat	Submandibular gland	retrograde ductal injection	CFTR
6	SJL/J mouse (ex vivo)	inguinal and popliteal LN cells (enrich in lymphoblasts)	adoptive transfer experimental autoimmune encephalomyelitis (EAE) model in irradiated mice/ i.p. injection of NR4A2 siRNA-treated LN cells	orphan nuvlear receptor NR4A2
7	Mouse		intravenously injection	short poly I:C , long poly I:C(purpose :dsRNA-induced IFN-β production)
8	Mouse	spinal cord	spinal cord injection	GlyT1, GlyT2, GlyRα3
9	Mouse	colon	intrarectall injection	Toll like receptor(TLR)-2
10	Mouse	spinal cord	spinal cord injection	GlyR α3
11	Mouse	i.d transplanted tumor (human glioblastoma cell), intracranial transplanted tumor (human glioblastoma cell)	intratumor injection	Eg5
12	Mouse	spleen	tail vein	Histamine H4 Receptors
13	Mouse		Intraperial injection	Stat1

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in vivo

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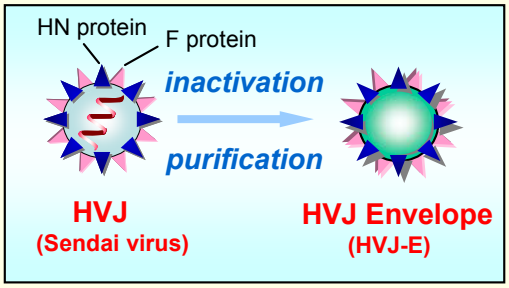
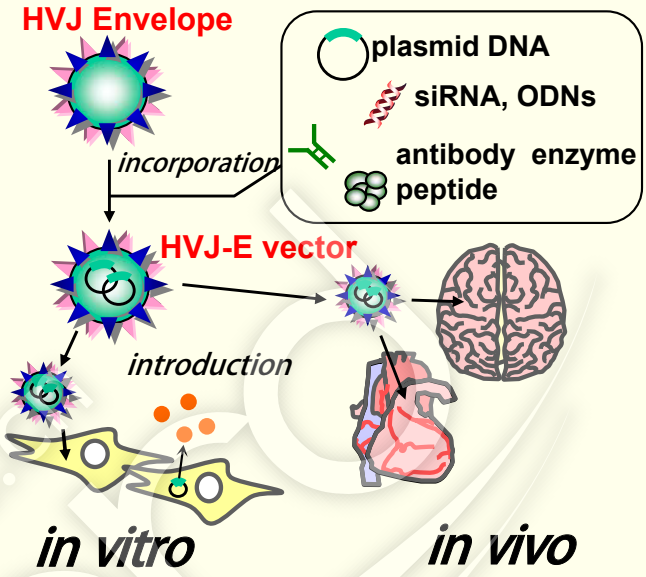
What is HVJ Envelope (HVJ-E) ?



HVJ Envelope (HVJ-E) is a purified product prepared through complete inactivation of Sendai virus (HVJ: Hemagglutinating Virus of Japan). It is a vesicle in which only the cell membrane-fusing capability of the envelope protein is retained.

Transfection using HVJ-E vector



Reference:
Kaneda, Y., *et al.*: Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. ***Molecular Therapy***, 6, 219-226 (2002)

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