

For research use

HVJ Envelope transfection KIT

GenomONETM-Neo EX

siRNA data sheet

 **ISHIHARA SANGYO KAISHA, LTD.**

1-3-15, Edobori, Nishi-ku, Osaka 550-0002 JAPAN

URL: <http://www.iskweb.co.jp/hvj-e/>

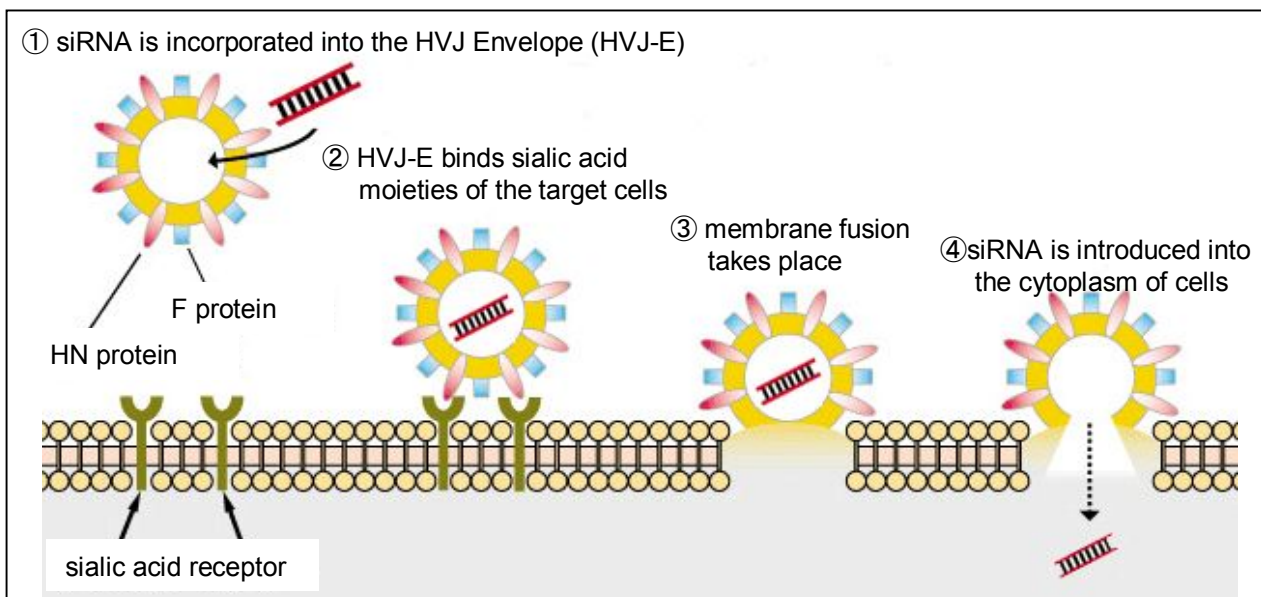
E-MAIL: HVJ-E@iskweb.co.jp

- Knock-down efficiency is too low to permit functional analysis, although siRNA has been introduced into the cells
- Cytotoxicity is too high to permit accurate evaluation
- Require a tool for siRNA transfer which can be used *in vivo*



Please try using ***GenomONE™-Neo***
in any of the following cases

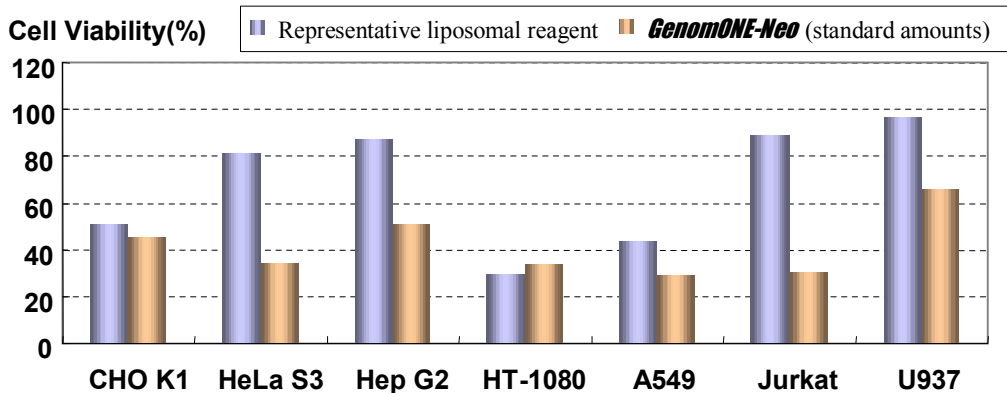
- **A totally novel means of direct introduction of siRNA into the cytoplasm**
 - This series of products enables efficient transfer of siRNA into cells by utilizing the membrane fusion capability of the envelope protein of Sendai virus(HVJ).
- **It can be used with diverse types of cells, ranging from adherent and non-adherent cell lines in culture to cells *in vivo* (in experimental animals).**
- **It yields rapid siRNA transfection into various and numerous samples.**
 - High-throughput RNAi library screening is possible with it.
 - One kit (Cat#.GN004EX) allows transfection into sixteen 24-well plates (about 400 wells) (if the standard siRNA protocol recommended by our company is followed).



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

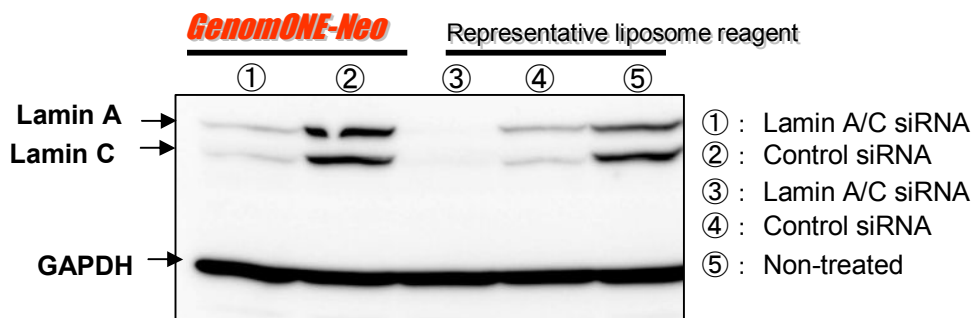
Comparison of activity of introduced siRNA between *GenomONE™* and liposomal reagents

【Example 1】Suppression of cell proliferation by Eg5 knock down



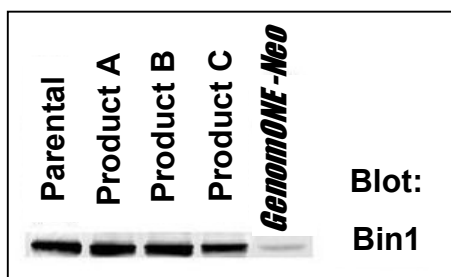
Eg5-siRNA was incorporated in each reagent to a final concentration of 50 nM in a 96-well plate. Cells of various lines were treated with the reagents. Forty-eight hours later, the percentage of viable cells was measured by WST-1 assay. Eg5 knock-down resulted in suppression of cell proliferation and induced apoptosis. The finding of a lower percentage of viable cells indicated stronger Eg5 knock-down effects.

【Example 2】Introduction of Lamin A/C siRNA (HUVEC)



Lamin A/C-targeted siRNA and control siRNA (each at a final concentration of 50 nM) were introduced into HUVEC, followed 48 hours later by Western blot assay. When *GenomONE-Neo* was used, specific knock-down of lamin A/C protein was noted (lane ①). On the other hand, when representative existing liposomal reagents were employed, non-specific suppression of lamin A/C expression was noted (lane ④).

【Example 3】Introduction of Bin-1 siRNA(C2C12)



※Parental : control cells without siRNA treatment

Twenty-four hours after induction of differentiation, C2C12 cells (mouse myoblast cell line) were transfected with Bin 1 siRNA using conventional representative siRNA transfection reagents (products A, B, and C) and *GenomONE-Neo*. Other products failed to exert sufficient knock-down effects, demonstrating the superiority of *GenomONE-Neo* to these products.

【Data】 Dr. Chie Kojima* and Dr. Hisataka Sabe

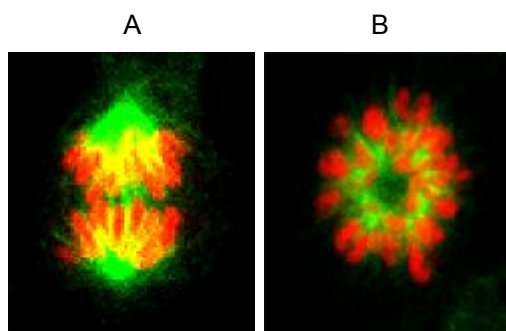
Department of Molecular Biology, Osaka Bioscience Institute, Japan.

* Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Japan.

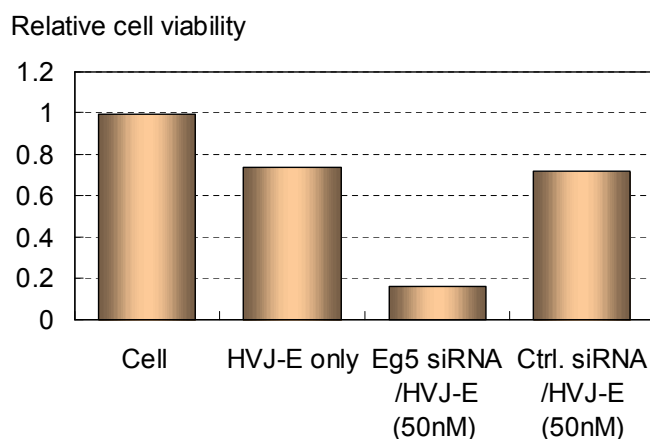
【Related article】 C. Kojima et al. *EMBO Journal*, 23, 4413-4422 (2004).

Suppression of HT1080 cell proliferation by Kinesin Eg5 knock-down

① Disturbance of orientation of spindle fibers following Eg5 knock-down

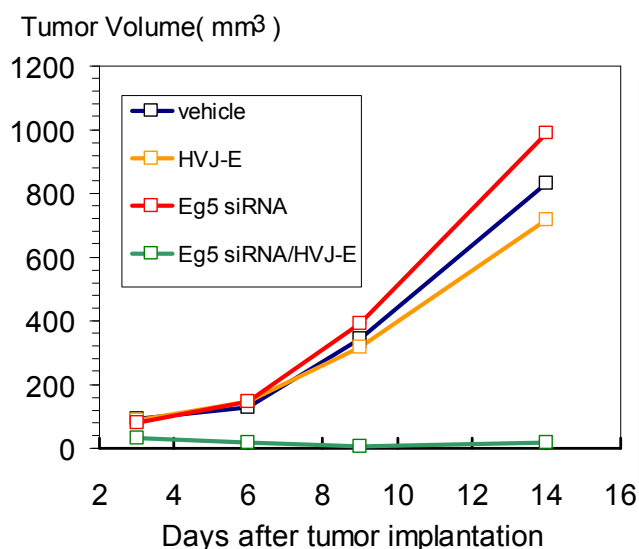


② Induction of apoptosis by Eg5 knock-down



Eg5 siRNA (50 nM) was introduced with *GenomONE-Neo* into HT-1080 cells. Twenty-four hours later, the cells were observed under a confocal laser scanning microscope. In the control cells with siRNA introduction (①-A), normal orientation of spindle fibers (α -tubulin; green) and normal division of chromosomes (DNA; red) were noted during cell division. In the cells with Eg5 siRNA introduction, abnormal spindle formation and abnormal chromosome segregation were noted during metaphase of the cell cycle (①-B), and apoptosis was induced as a result of suppression of cell division (②).

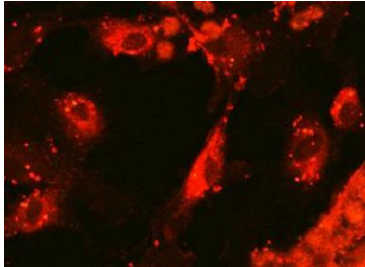
③ Suppression of subcutaneous tumor growth in mice by Eg5 knock-down (nude mice with subcutaneous implantation of HT-1080 cells)



Eg5-targeted siRNA (56 nM) was introduced into HT-1080 cells in a 10 cm diameter dish, using *GenomONE-Neo*. The cells were harvested after 4 hours of incubation and were subcutaneously implanted in BALB/c mice (5×10^5 cells/mouse). Measurement of tumor volume at multiple points of time after implantation revealed almost complete suppression of tumor formation. In mice treated with Eg5 siRNA alone, tumor formation was only slightly suppressed. These findings suggest that HVJ-E is useful as a means of introducing siRNA into cells.

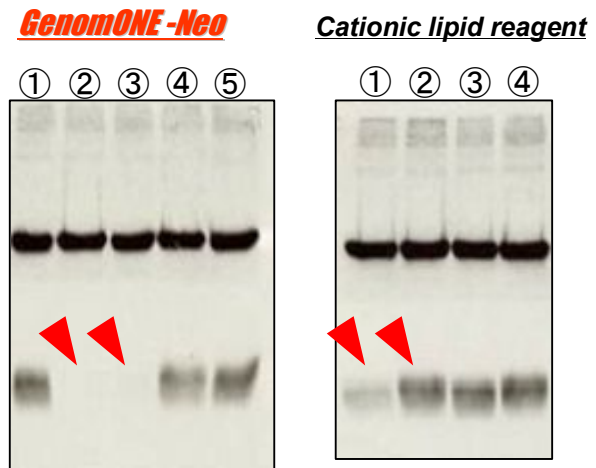
Phospholamban (PLB) knock-down in primary rat myocardial cell cultures

① Transfection with Cy3-labeled siRNA



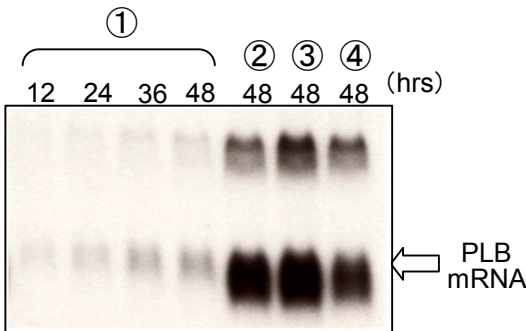
siRNA (30 nM) was introduced into neonatal rat myocardial cells (primary cultures).

③ Western blot assay



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

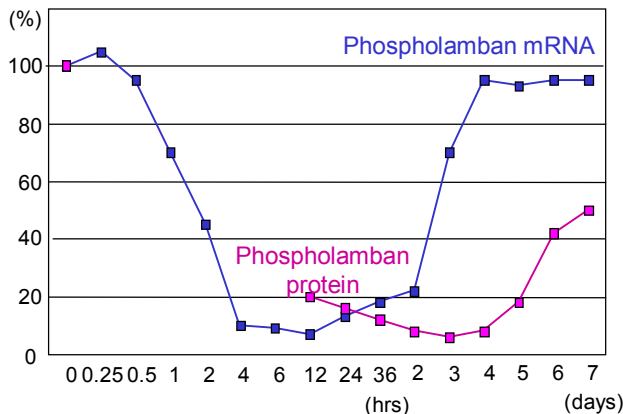
② Northern blot assay



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

The efficiency of siRNA transfer into cells was high (80-100%) with both reagents. However, compared to cationic lipid, **GenomONE-Neo** induced knock-down of the target protein more efficiently at lower concentrations of siRNA.

④ Long-lasting activity of siRNA.



Transfection with PLB-specific siRNA using **GenomONE-Neo** resulted in 80% or greater suppression of expression of PLB protein from 1 day to 5 days after transfection.

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

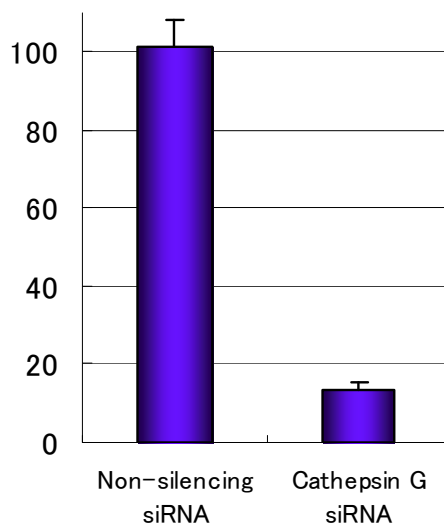
【Related article】 A. Watanabe *et al.* *J. Mol. Cell. Cardiol.*, **37** (3), 691-698 (2004)

RNAi in difficult-to-transfect U937 cells

Although siRNA transfection to U937 human leukemic monocytic cells is reported to be difficult even with liposomal reagents and electroporation, this is an example of an experiment involving effective introduction of siRNA by means of **GenomONE-Neo**, with a strong knock-down effect of over 85% obtained.

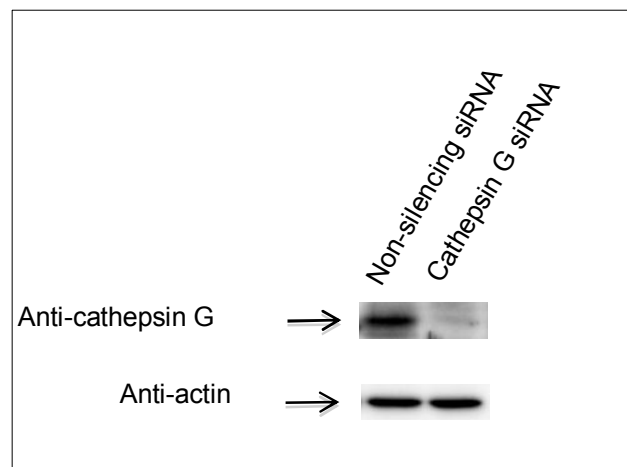
- ▶ U937 cells were collected 72 hours after siRNA transfection, and changes in amounts of mRNA and protein were evaluated by real-time PCR and Western blotting, respectively.

Real-time PCR



Introduction of Cathepsin G-specific siRNA reduced the mRNA level for cathepsin G up to approximately 14%.

Western blotting



Introduction of Cathepsin G-specific siRNA reduced the protein level for cathepsin G up to approximately 15%.

- ▶ Since **GenomONE-Neo** exhibited higher transfection efficiency and had lower cytotoxicity than the standard method with liposomal reagents or electroporation, sufficient knock-down effect was obtained.

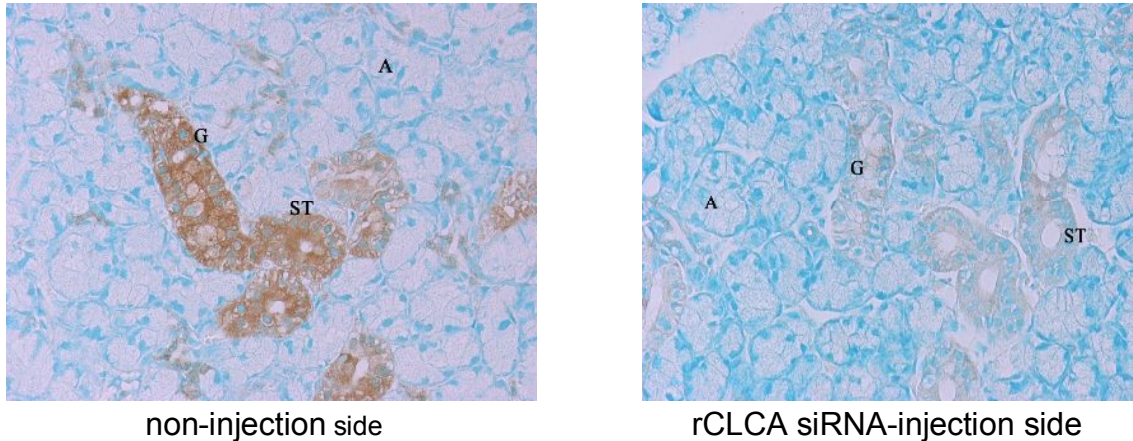
[Data] Dr. Y. Tsuchiya (Department of Hygienic Chemistry, Showa Pharmaceutical University).
Present address: Department of Degenerative Neurological Diseases,
National Institute of Neuroscience, National Center of Neurology and Psychiatry.

[Related article]

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).

Retrograde Injection of siRNA into Rat Submandibular Gland (*in vivo*)

Specific suppression of expression of Ca²⁺-dependent Cl⁻ channel protein (rCLCA) Immunostaining



rCLCA siRNA (2 nmol) was injected in retrograde fashion into the submandibular glands of rats, using **GenomONE-Neo** (2 Assay Unit). Forty-eight hours later, specific suppression of expression of Cl⁻ channel protein was demonstrated on the siRNA-injection side by immunostaining.

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

Suppression of Cl⁻ reabsorption in submandibular gland

Treatment group	Electrolyte concentrations in final saliva (mM)		
	Na ⁺	K ⁺	Cl ⁻
rCLCA siRNA-injection side (n=11)	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
scrambled siRNA-injection side (n=9)	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) was injected in retrograde fashion into the submandibular glands of rats, using **GenomONE-Neo** (2 Assay Unit). Forty-eight hours later, secreted saliva was collected while a drug stimulating salivary release (pilocarpine) was administered. Analysis of the concentrations of electrolyte in the saliva collected revealed a significantly higher Cl⁻ level in the group with injection of rCLCA siRNA than in the control group (scrambled siRNA-injection group), confirming the efficacy of rCLCA siRNA injection in specifically suppressing Cl⁻ reabsorption. Reabsorption of Na⁺ and K⁺ remained unaffected.

Injection of siRNA, targeted at cystic fibrosis transmembrane conductance regulator (CFTR), also resulted in specific suppression of Cl⁻ reabsorption.

[Data] Dr. Kazunari Ishibashi
Department of Functional Bioscience, Fukuoka Dental College (Japan).

[Related article] 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	Brp2
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 α 2
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 α 2
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 nuclear 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, GlyR α 3
9	Mouse	colon	intrarectall injection	Toll like receptor(TLR)-2
10	Mouse	spinal cord	spinal cord injection	GlyR α 3
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
14	Rat		Intrathecal(i.t) injection	CaV 3.2
15	Mouse	uterus	uterine cavity	RCAS1

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in vivo

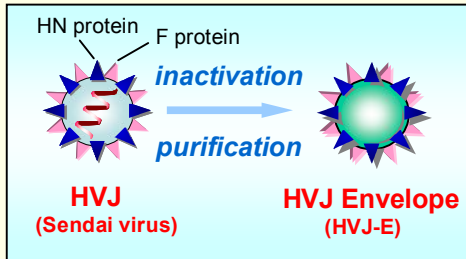
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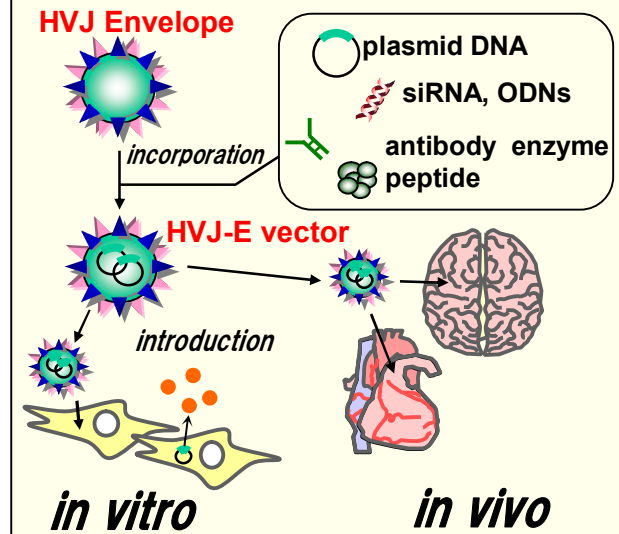
NOTES

■ 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)

ISK ISHIHARA SANGYO KAISHA, LTD.

1-3-15, Edobori, Nishi-ku, Osaka 550-0002 JAPAN

URL: <http://www.iskweb.co.jp/hvj-e/>

E-MAIL: HVJ-E@iskweb.co.jp