

For research use

ver.1.1ENG

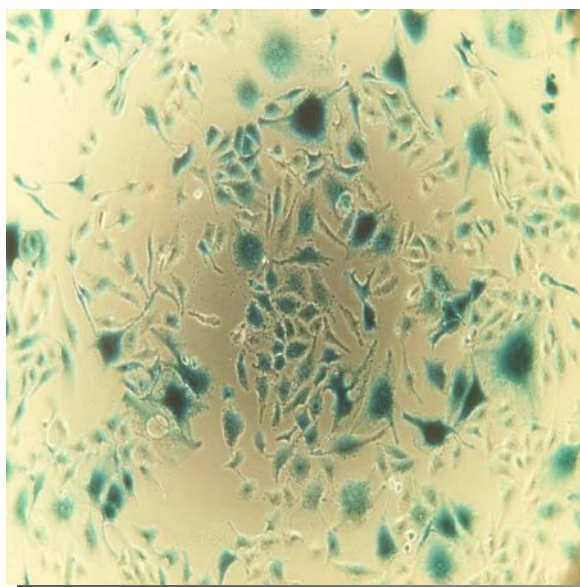
*HVJ Envelope Transfection Kit*

***GenomONE™ - Neo EX***

***GenomONE™ - CAb EX***

# **Data Sheet for Protein Delivery**

*Efficient Delivery of Functional Proteins, Peptides and Antibodies  
into Living Cells*



***ISK*** ISHIHARA SANGYO KAISHA, LTD.

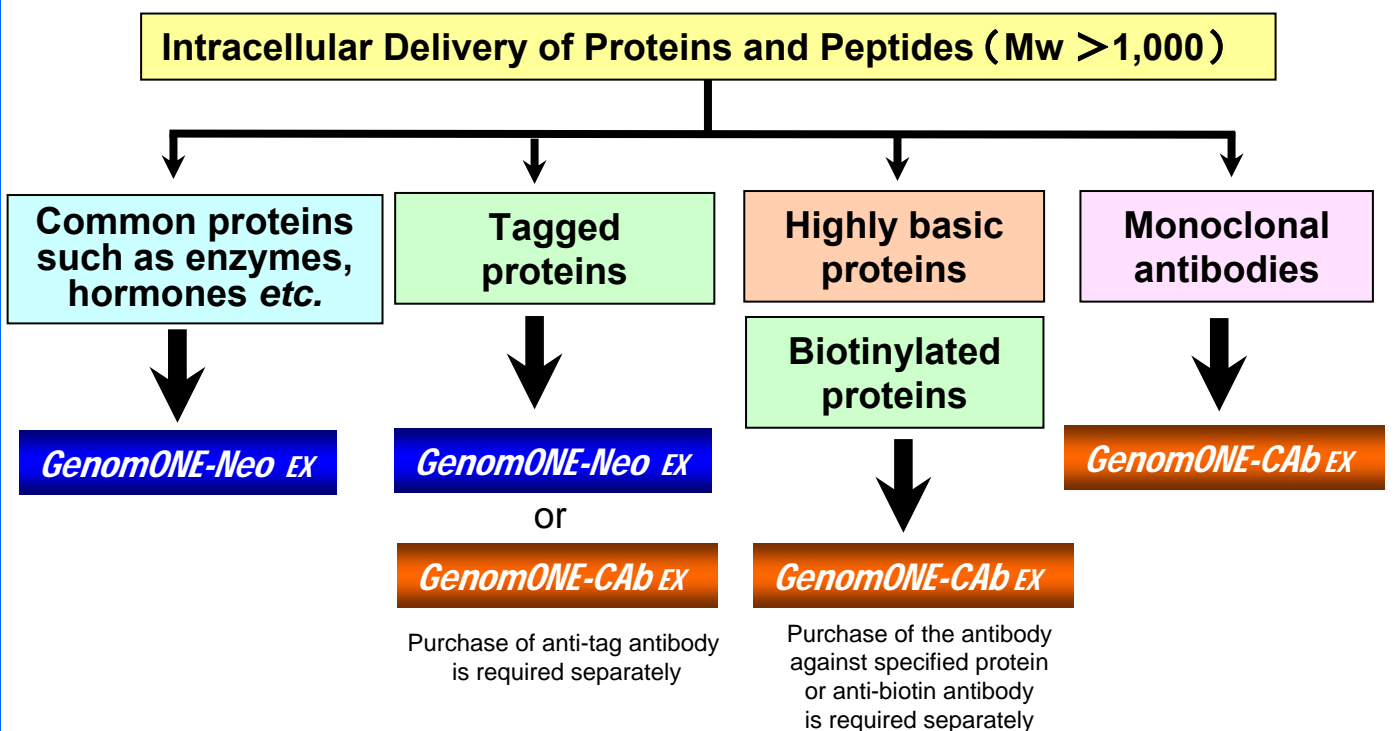
1-3-15, Edobori, Nishi-ku, Osaka 550-0002 JAPAN

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

E-MAIL: [HVJ-E@iskweb.co.jp](mailto:HVJ-E@iskweb.co.jp)

# Total Solutions of Protein Delivery by the GenomONE Series

- ▶ *GenomONE series is a novel transfection vector kit, which employs the membrane fusion ability of the HVJ Envelope*
- ▶ *Efficient and useful tool to analyze cellular functions by introducing functional proteins into living cells*
- ▶ *Applicable for in vitro and in vivo experiments*



**<Examples> p 3~5**

Luciferase  
 β Galactosidase  
 RNase T1  
 RNase 1  
 SOD  
 OVA  
 VEGF receptor 2 (13 aa)  
 nNOS  
 Pasteurella multosida toxin  
 HA-Avidin  
 FITC-BSA  
 Alexa 488-BSA  
 FITC-Insulin  
 Alexa 488-Insulin  
 FITC-Lysozyme  
 etc.

**<Examples> p 2**

Cre-recombinase (Flag-Cre-His)  
 Myc-β Gal  
 HA-Avidin  
 etc.

**<Example>**

HA-Avidin  
 Biotinylated-β Gal

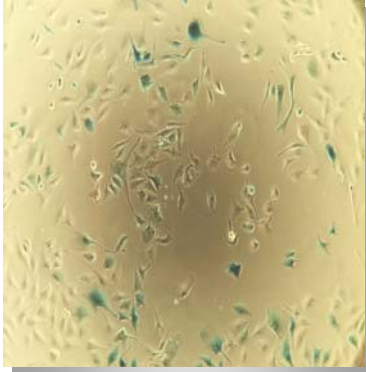
**<Examples> p 5**

anti-NPC  
 anti-α tubulin  
 anti-α adaptin  
 anti-Lamp-1  
 anti-NF κ B  
 anti-STAT-1  
 anti-IRF-1  
 Control IgG  
 (mouse, rat, human, rabbit, goat),  
 Alexa 488 anti-α tubulin  
 etc.

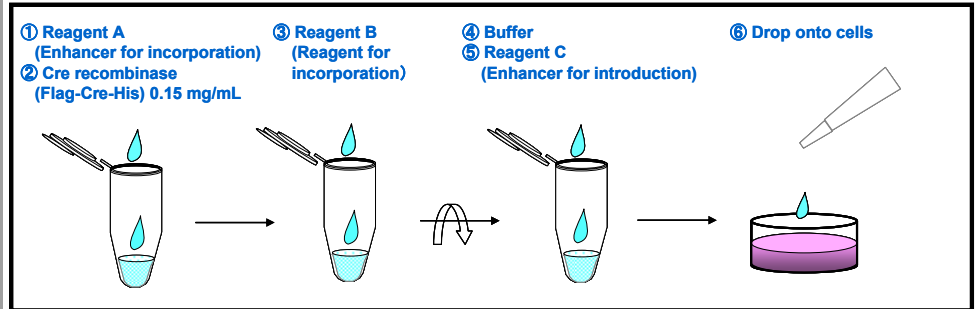
For further information on the specifications of *GenomONE-Neo* and *GenomONE-Cab*, please contact us by e-mail: HVJ-E@iskweb.co.jp

# ▶ $\beta$ -Galactosidase expression triggered by intracellular delivery of Cre recombinase (Flag-Cre-His) (Performance compared with other protein delivery reagents)

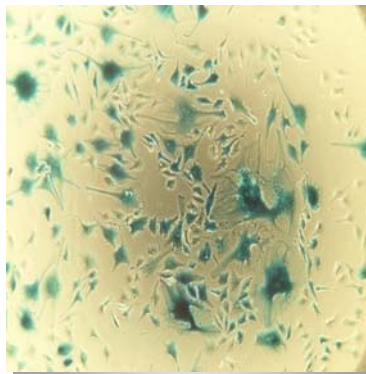
## *GenomONE-Neo*



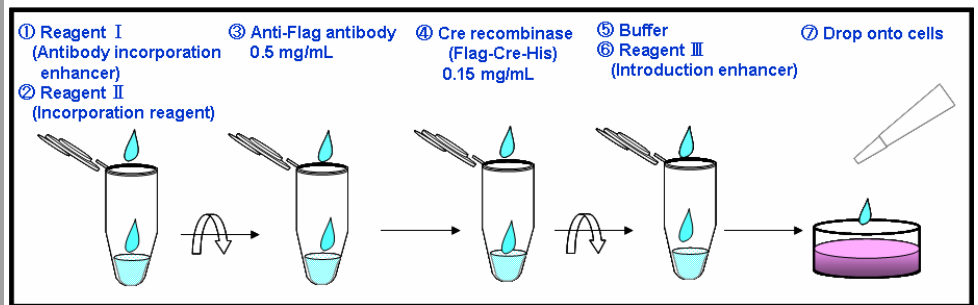
Cre recombinase was incorporated into HVJ-E particles using *GenomONE-Neo*, and then introduced into cells. Cells expressing active  $\beta$ -Gal molecules through processing by introduced Cre were observed.



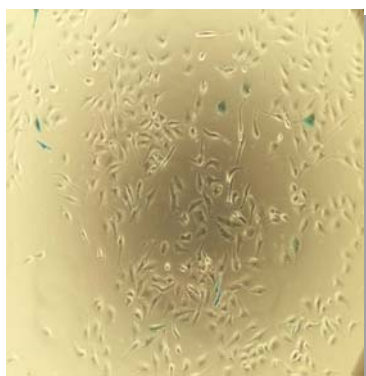
## *GenomONE-CAb*



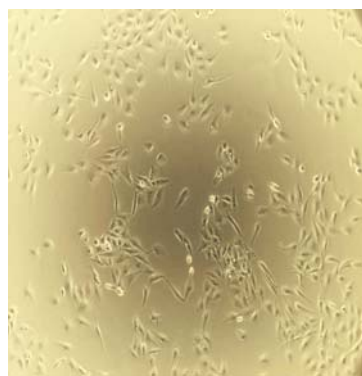
Cre recombinase was incorporated into HVJ-E particles in which an anti-Flag antibody had been previously encapsulated using *GenomONE-CAb*. This procedure facilitated the incorporation of Cre proteins, which increased the introduction efficiency into the cells.



## Product B



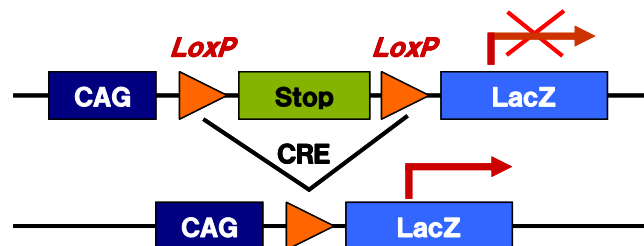
## Product C



## Product V

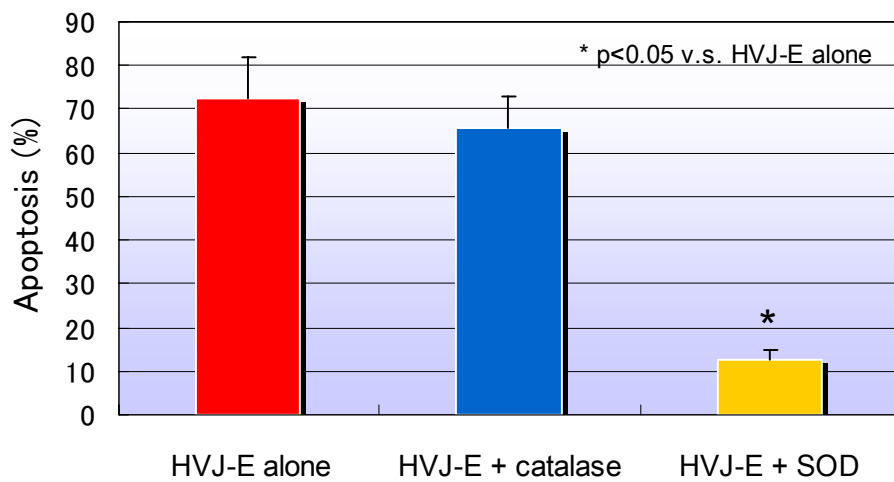


## 【Experiment】



By introducing Cre recombinase into cells, loxP sites inserted in the genome sequence are deleted and Lac Z gene expression is induced. Cre recombinase was delivered into 2-2 cells ( $1.5 \mu\text{g}/\text{well}$ ) and incubated for 24 hours. X-Gal reagent was then added and the cells incubated overnight followed by evaluation of activity in the cells. Higher expression of  $\beta$ -Gal activity was obtained when *GenomONE* was used as compared to three other reagents.

▶ **Suppression of radiation-induced apoptosis following SOD introduction into mouse primary macrophages**



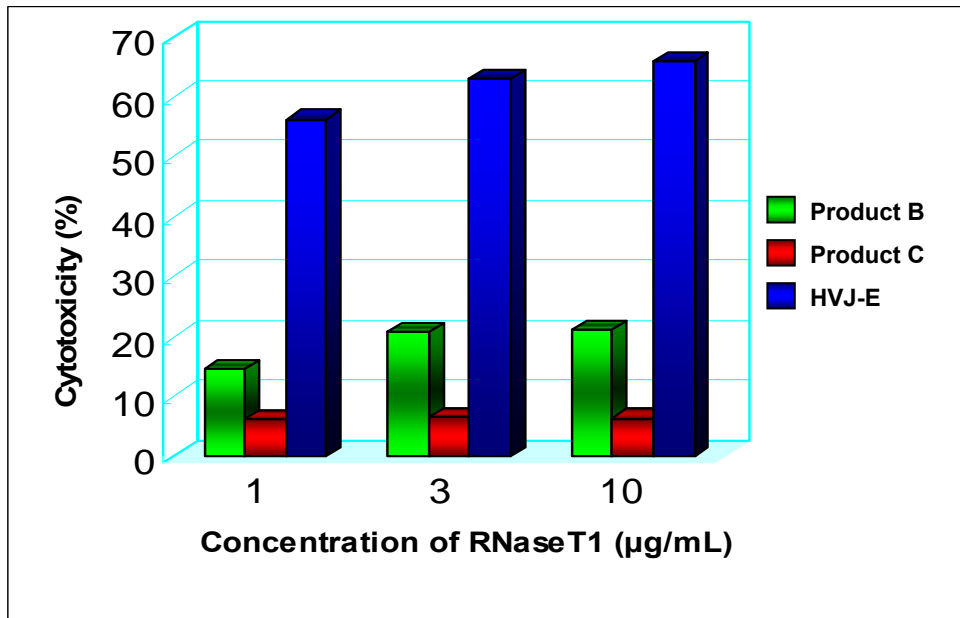
SOD was introduced into mouse peritoneal macrophages, using *GenomONE* (HVJ-E). One hour later, the macrophages were gamma-irradiated. Radiation-induced apoptosis was suppressed in the macrophages with SOD introduction. Introduction of catalase did not suppress radiation-induced apoptosis. Suppression of radiation-induced apoptosis was thus shown to be specific to SOD.

[Data] Dr. Y. Kubota *et al.*, National Institute of Radiological Sciences (Japan).

[Related article] Kubota Y. *et al.*: *Int. J. Radiat. Biol.*, **81**, 459-472 (2005).

▶ **Tumor cell death induced by intracellular delivery of RNase T1 (SAS cells)**

(Performance compared with other protein delivery reagents)



RNaseT1 was delivered into SAS cells (tongue derived squamous cell carcinoma) using *GenomONE* (HVJ-E) or two alternative protein delivery reagents. Twenty hours later, cellular metabolic activity (cytotoxicity) was assessed by means of a WST-1 assay.

RNaseT1 alone did not induce tumor cell death because of its inability to permeate the cell membrane (data not shown). In contrast, RNaseT1 incorporated in HVJ-E induced cytotoxicity in a concentration dependent manner, suggesting that intact enzyme was delivered into the cytoplasm without losing its activity. Cytotoxic activity induced by RNaseT1/HVJ-E was higher than those induced by the other two reagents.

[Related article] Yuki S. *et al.*: *Eur. J. Biochem.*, **271**, 3567-3572 (2004)

# ► Delivery of $\beta$ -Galactosidase into NIH-3T3 cells

(Performance compared with other protein delivery reagents)

*HVJ-E vector system bypasses degradation or denaturation by lysosomal enzymes, making it easy to uniformly deliver the bioactive proteins into the cytoplasm*



Fig. 1 Delivery of  $\beta$ -Gal protein (Phase contrast)

$\beta$ -Gal was delivered into cells using each reagent. After incubation for four hours, non-specifically bound  $\beta$ -Gal was degraded by trypsin treatment. Cells were then treated with X-Gal reagent to detect  $\beta$ -Gal-expressing cells.

Uniform distribution of  $\beta$ -Gal was observed when *GenomONE* (HVJ-E) was used, whereas delivery of protein using the other two reagents was not uniform. Aggregation of delivered protein in the cells was apparent when Product B was used.

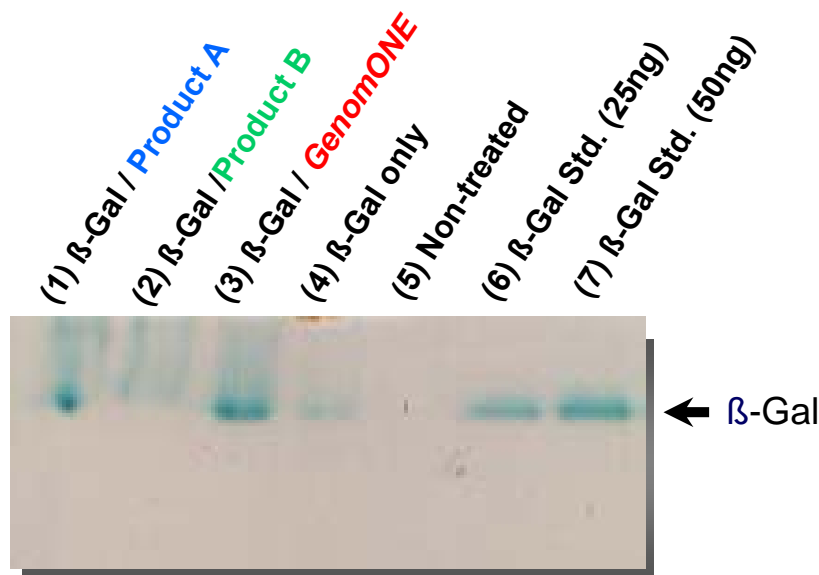


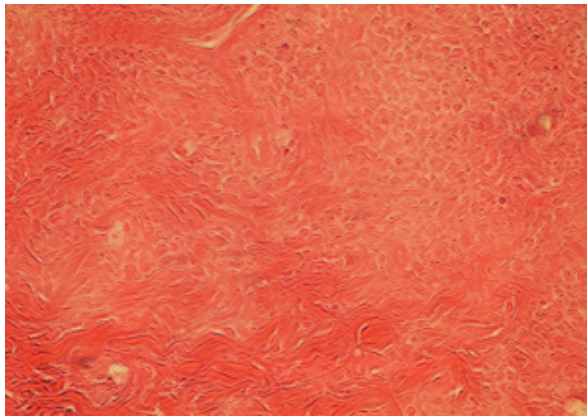
Fig. 2 PAGE analysis (non-denaturing condition) of  $\beta$ -Gal protein extracted from cells (X-Gal staining)

Four hours after intracellular delivery of  $\beta$ -Gal, cells were collected and lysed by freezing and thawing, and then analyzed by PAGE under non-denaturing conditions (without SDS) followed by staining with X-Gal reagent. X-Gal stained-positive clear band (Lane 3) with the same molecular weight as standard  $\beta$ -Gal (Lane 6, 7) was detected when *GenomONE* (HVJ-E) was used. This result suggests that  $\beta$ -Gal incorporated in the HVJ-E was delivered into cytoplasm without degradation.

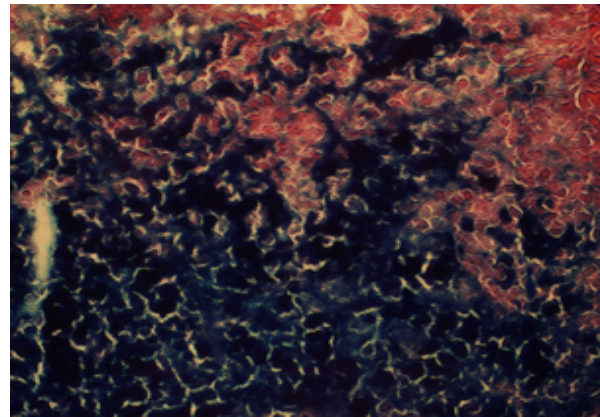
In contrast,  $\beta$ -Gal molecules extracted from cells in which cationic lipid-based two other reagents were used for delivery exhibited smeared patterns (Product A: Lane 1, Product B: Lane 2), suggesting that the molecules could be degraded during the introduction step.

Unlike other lipid-based reagents, HVJ-E delivers the specified proteins directly into the cytoplasm through membrane fusion. Therefore, the HVJ-E system has an advantage that it resists degradation by lysosomal enzymes<sup>4</sup>

▶ **Delivery of  $\beta$ -Galactosidase into intradermally transplanted Colon 26 tumor cells in mouse**



$\beta$ -Gal alone

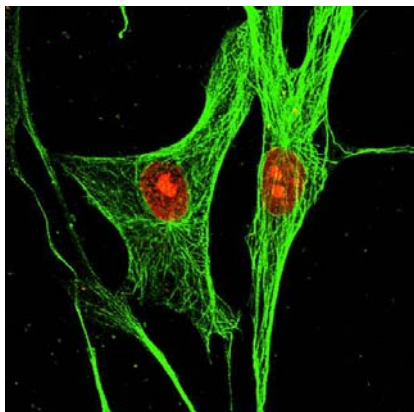


$\beta$ -Gal/*GenomONE-Neo*

(X-Gal staining)

Colon 26 mouse colorectal carcinoma cells were intradermally implanted into the back of 5-week-old mice. One week later,  $\beta$ -Gal incorporated in the HVJ-E (*GenomONE-Neo*) or  $\beta$ -Gal solution alone was injected into the tumors. Twenty four hours after injection, tumors were excised and their frozen sections were prepared followed by staining with X-Gal reagent. Strong X-Gal staining-positive cells were observed when *GenomONE-Neo* was used, suggesting that intact  $\beta$ -Gal molecules were delivered into the cytoplasm without losing their enzyme activities (Right). In contrast, almost all of the cells stained negative when  $\beta$ -Gal alone was injected without HVJ-E (Left).

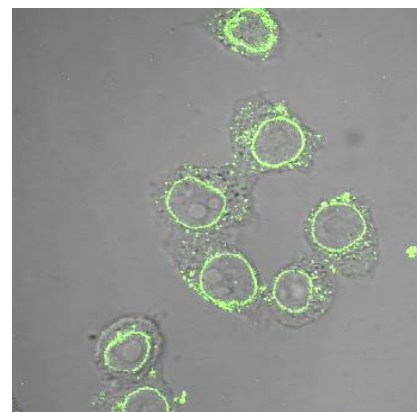
▶ **Antibody delivery into living cells using *GenomONE-CAb***



**Introduction of anti- $\alpha$ -tubulin antibody\* into Hs68 cells**

Nucleus of each cell was stained with SYTO 82 (red)

\* Monoclonal anti- $\alpha$ -tubulin, Clone DM1A  
Mouse IgG1 (SIGMA, T6199)



**Introduction of anti-NPC antibody\* into HeLa S3 cells**

\* Monoclonal anti-Nuclear Pore Complex Proteins  
Clone 414, Mouse IgG1 (SIGMA, N8786)

Two hours after antibody delivery, cells were fixed and treated with Alexa Fluor 488-goat anti-mouse IgG F(ab')<sub>2</sub> fragment (Invitrogen A11017), thereafter observed by confocal laser scanning microscopy.

## ▶ Published researches using GenomONE

### Protein/Peptide delivery (*in vitro*)

First Author	Reference	PubMed ID	Cell	Protein/Peptide
S. Yuki	<i>Eur. J. Biochem.</i> , 271, 3567-3572 (2004)	15317592	K562, SAS, G402, BHK-21	RNaseT1
Y. Kubota	<i>Int. J. Radiat. Biol.</i> , 81, 459-472 (2005)	16249161	Mouse primary macrophage	SOD, FITC-BSA (Efficiency of delivery; 90%)
T. Matsumoto	<i>EMBO J.</i> , 24, 2342-2353 (2005)	15962004	HUVEC	VEGF receptor 2 (13 aa peptide)
K. Kitadokoro	<i>PNAS</i> , 104, 5139-5144 (2007)	17360394	Swiss 3T3	PMT (Pasteurella multocida toxin)
T. Tani	<i>Cloning and Stem Cells</i> , 9, 267-280(2007)	17579559	Bovine cumulus cell	TCTP (Phosphorylated transcriptionally controlled tumor protein) peptide (Efficiency of delivery of FITC-TCTP; 99%)

### Protein delivery (*in vivo*)

First Author	Reference	PubMed ID	Target organ / tissue	Protein
K. Owada-Makabe	<i>Neurosci. Lett.</i> , 378, 18-21 (2005)	15763165	Rat brain (nucleus tractus solitarius)	β-galactosidase
E. Yasuoka	<i>J. Mol. Med.</i> , 85, 279-288 (2007)	17072578	Mouse nasal cavity	Alexa488-OVA (ovalbumin), Alexa488-BSA

### Antibody delivery into living cells (*in vitro*)

First Author	Reference	PubMed ID	Cell	Monoclonal antibody
Y. Kondo	<i>J. Immunol. Methods</i> , 332, 10-17(2008)	18221753	HeLaS3, Hs68, A549, SAS, HT1080, MCF-7, WI38, BNL-CL2, BHK-21, Raw264.7, Jurkat, P19, 3T3-L1	anti-NPC, anti-alpha tubulin, anti-alpha adaptin, anti-NFκB, Control IgG (mouse, rat, human, rabbit, goat)

## ▶ Efficiency of protein delivery using GenomONE

### Bovine Serum Albumin (Alexa488-labeled) delivery

Cell line	Cell type	Efficiency of protein delivery (ratio of fluorescence-positive cells/FACS analysis)
A7r5	Rat thoracic aortic smooth muscle	99%
Astrocyte	Mouse astrocyte	95%
B16-F1	Mouse melanoma	70%
BHK-21	Hamster kidney fibroblast	~100%
Colon-26	Mouse colon adenocarcinoma	97%
COS-7	Green monkey kidney fibroblast	75%
HEK293	Human kidney, transformed embryonic	91%
HeLa S3	Human cervical epithelial carcinoma	95%
HUH-7	Human hepatocarcinoma	93%
LLC-Mk2	Rhesus monkey normal kidney	97%
NIH-3T3	Mouse fibroblast	97%
PC-12	Rat pheochromocytoma (adrenal gland)	77%
Raw264.7	Mouse leukemic monocyte/macrophage	64%
SAS	Human tongue carcinoma	98%
FM3A	Mouse mammary carcinoma	99%
HL-60	Human promyelocytic leukemia	94%
Jurkat	Human T cell leukemia	90%
K562	Human myelogenous leukemia	99%
U937	Human leukemic monocyte	99%
AOSMC	Primary human aortic smooth muscle cells	98%
HUVEC	Primary human umbilical vein endothelial cells	89%
NHBE	Primary human bronchial epithelial cells	98%
SkMC	Primary human skeletal muscle cells	93%

### Insulin (FITC-labeled) delivery

FM3A	Mouse mammary carcinoma	99%
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### Insulin (Alexa488-labeled) delivery

BHK21	Hamster kidney fibroblast	~100%
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### Rabbit IgG (Alexa488-labeled) delivery

HL-60	Human promyelocytic leukemia	100%
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# What is HVJ Envelope (HVJ-E) ?

■ What is HVJ Envelope (HVJ-E) ?

HN protein F protein  
**inactivation**  
**purification**  
**HVJ (Sendai virus)** → **HVJ Envelope (HVJ-E)**

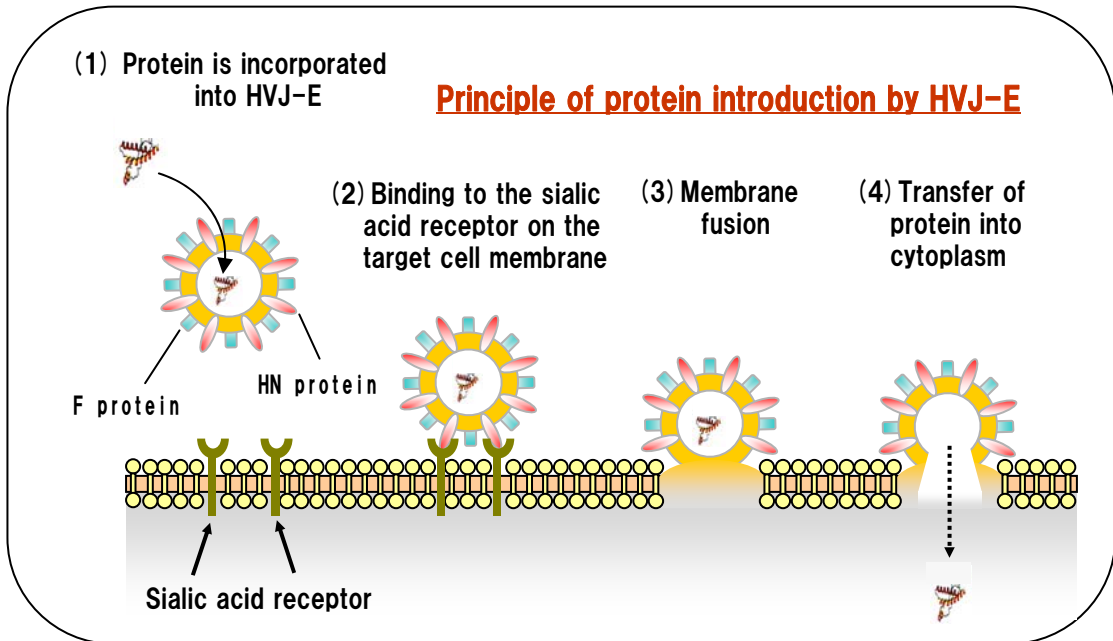
HVJ Envelope (HVJ-E) is a purified product prepared through **complete inactivation** of Sendai virus (HVJ: Hemagglutinating Virus of Japan). It is a vesicle in which only the cell membrane-fusing capability of the envelope protein is retained.

■ Transfection using HVJ-E vector

**HVJ Envelope**  
**incorporation**  
**HVJ-E vector**  
**introduction**  
**in vitro**      **in vivo**

- plasmid DNA
- siRNA, ODNs
- antibody enzyme
- peptide

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



**ISHIHARA SANGYO KAISHA, LTD.**

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