

For research use

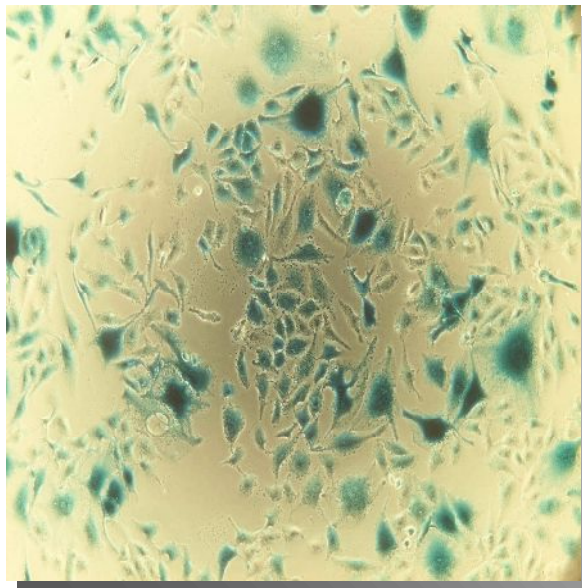
*HVJ Envelope Transfection Kit*

***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.

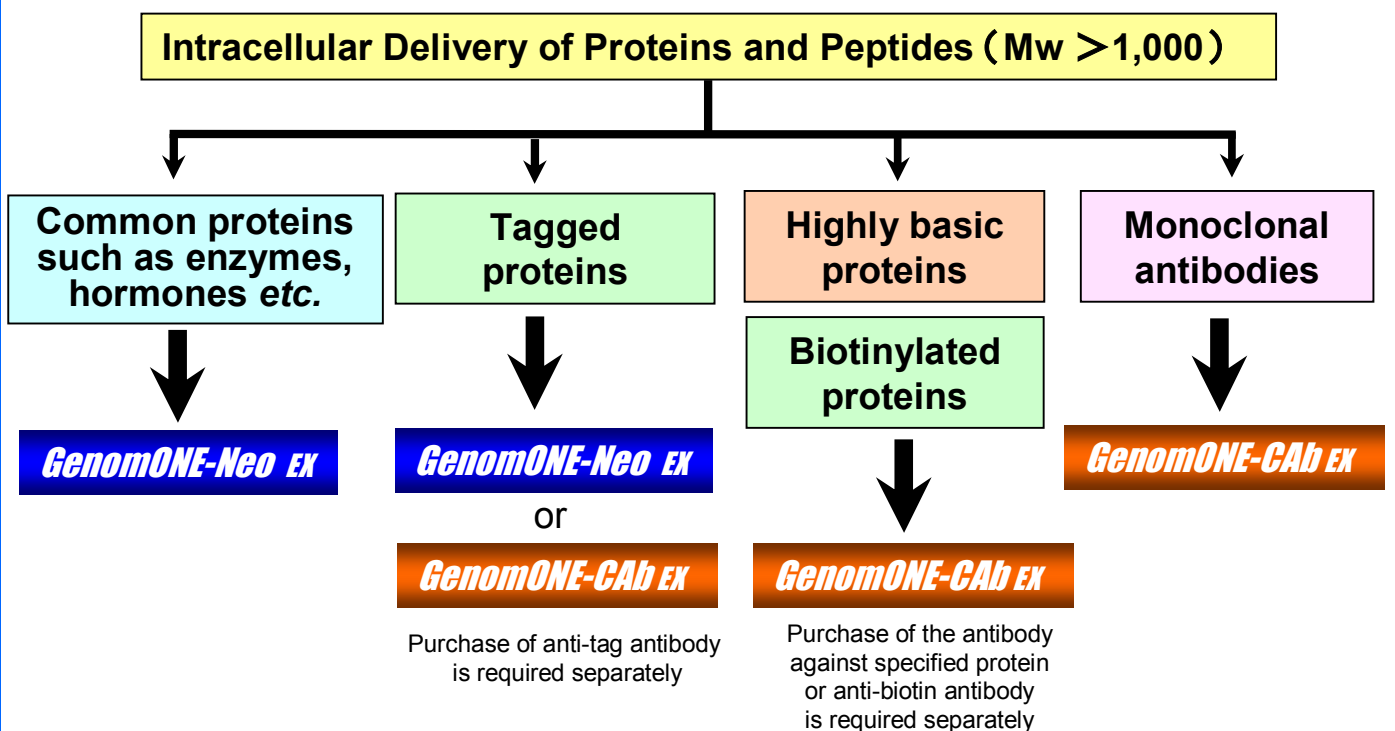
1-3-15, Edobori, Nishi-ku, Osaka 550-0002 JAPAN

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

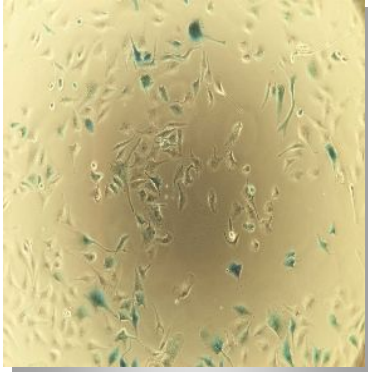
**<Examples> p 5**

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.

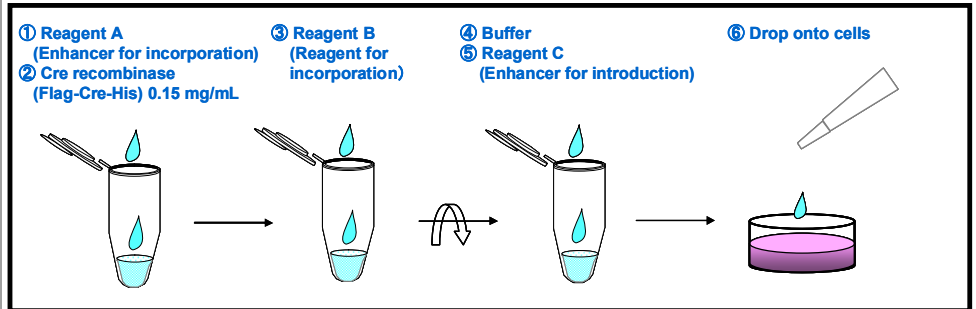
For further information on the specifications of *GenomONE-Neo* and *GenomONE-CAb*, please contact us by e-mail: HVJ-E@iskweb.co.jp

▶ **β-Galactosidase expression triggered by intracellular delivery of Cre recombinase (Flag-Cre-His)**  
 (Performance compared with other protein delivery reagents)

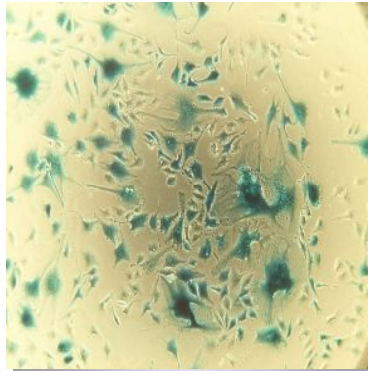
**GenomONE-Neo**



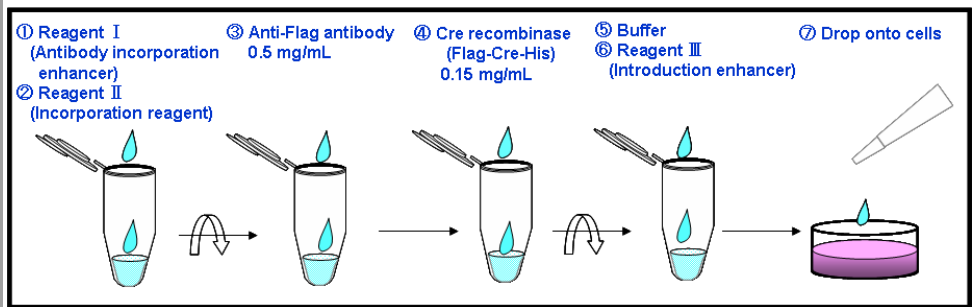
Cre recombinase was incorporated into HVJ-E particles using *GenomONE-Neo*, and then introduced into cells. Cells expressing active β-Gal molecules through processing by introduced Cre were observed.



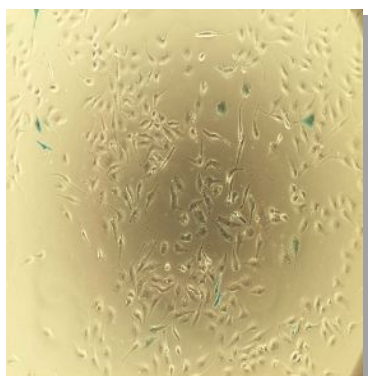
**GenomONE-CAb**



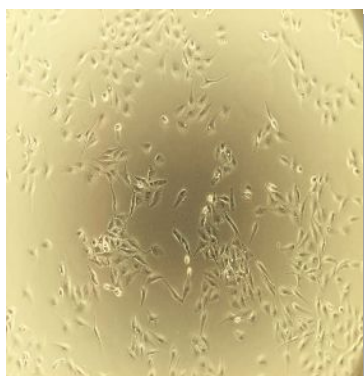
Cre recombinase was incorporated into HVJ-E particles in which an anti-Flag antibody had been previously encapsulated using *GenomONE-CAb*. This procedure facilitated the incorporation of Cre proteins, which increased the introduction efficiency into the cells.



**Product B**



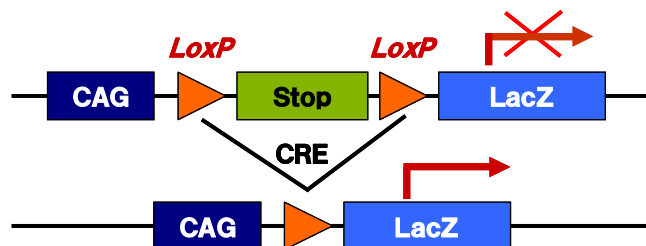
**Product C**



**Product V**

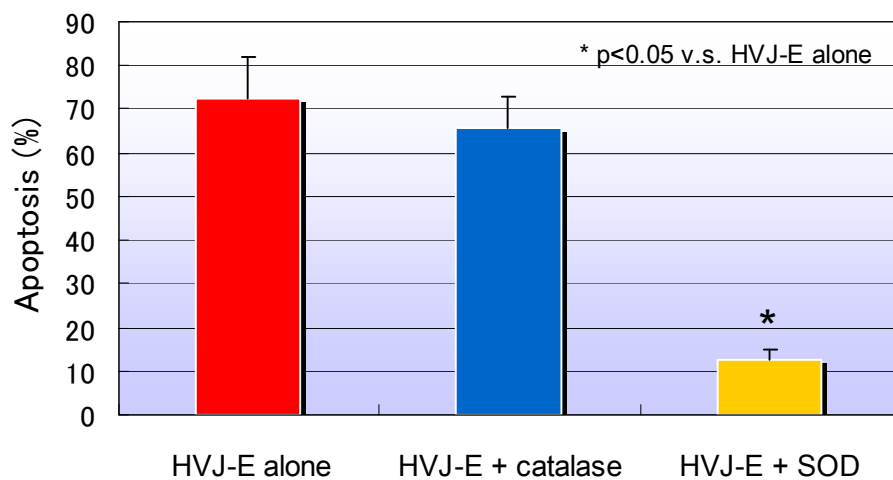


**【Experiment】**



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 μg/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 β-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



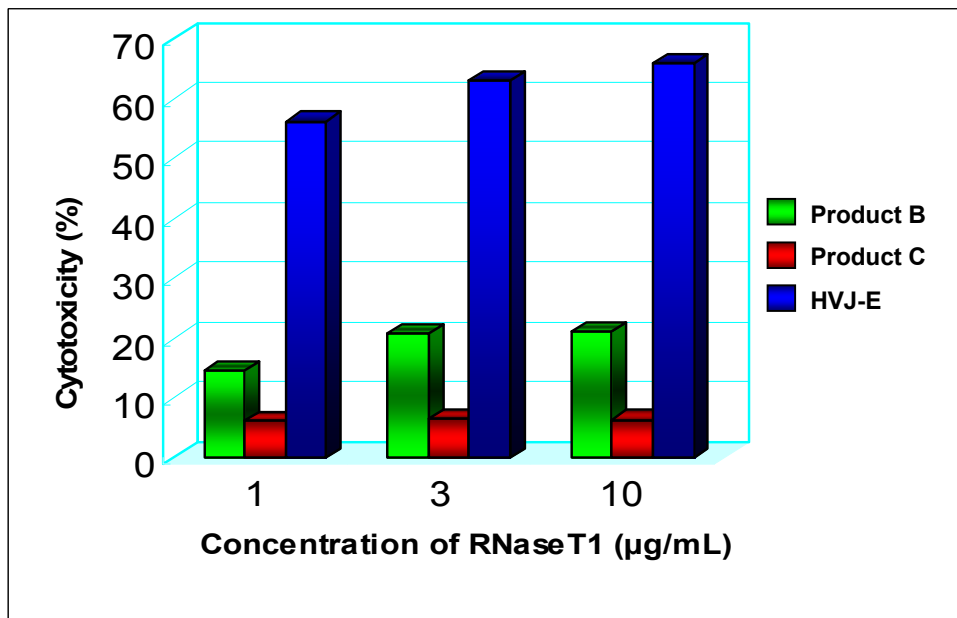
SOD was introduced into mouse peritoneal macrophages, using *GenomONE* (HVJ-E). One hour later, the macrophages were gamma-irradiated. Radiation-induced apoptosis was suppressed in the macrophages with SOD introduction. Introduction of catalase did not suppress radiation-induced apoptosis. Suppression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

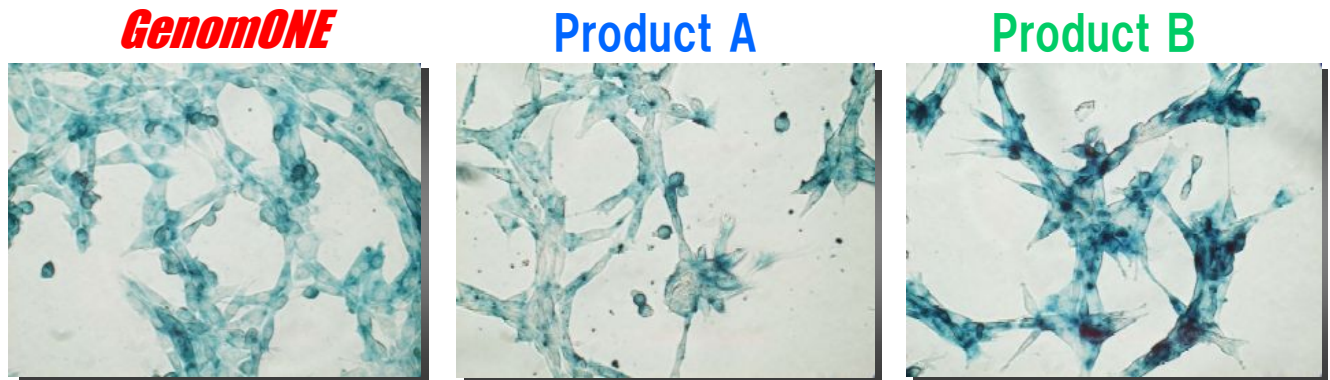


Fig. 1 Delivery of  $\beta$ -Gal protein (Phase contrast)

$\beta$ -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.

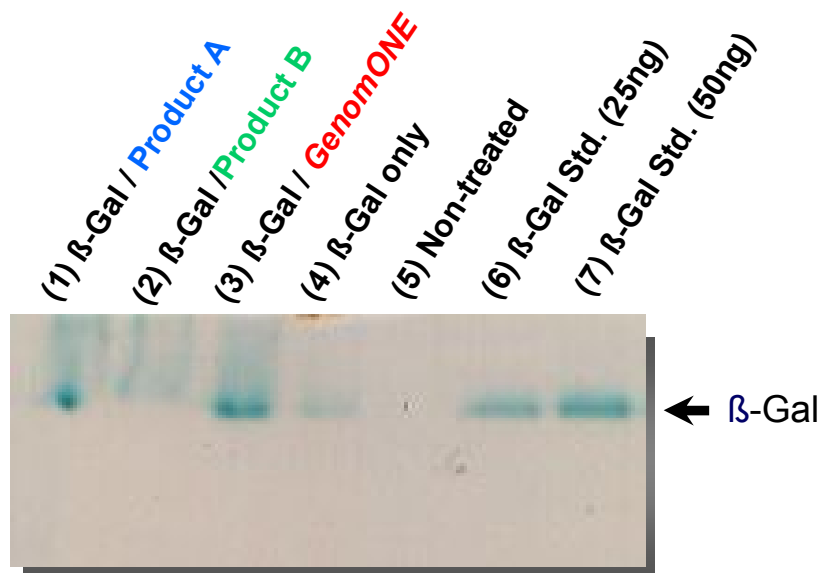


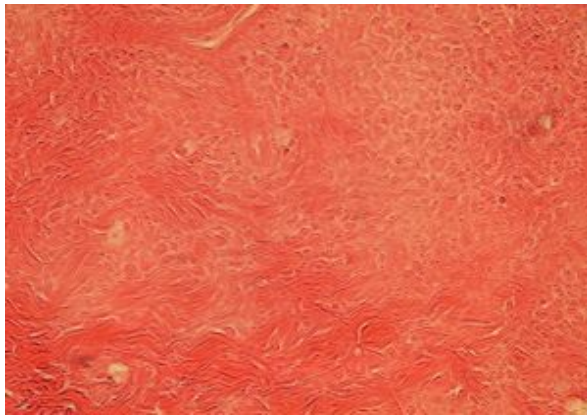
Fig. 2 PAGE analysis (non-denaturing condition) of  $\beta$ -Gal protein extracted from cells (X-Gal staining)

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.

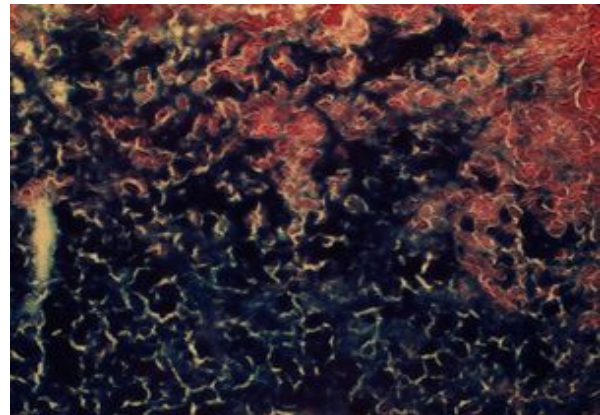
In contrast,  $\beta$ -Gal molecules extracted from cells in which cationic lipid-based two other reagents were used for delivery exhibited smeared patterns (Product A: Lane 1, Product B: Lane 2), suggesting that the molecules could be degraded during the introduction step.

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

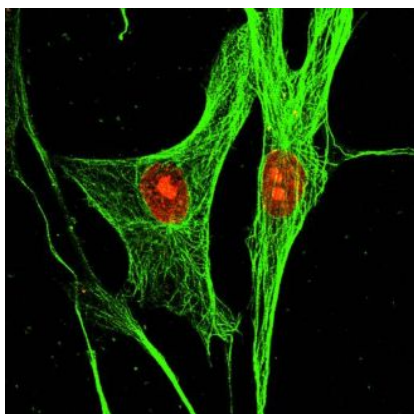


$\beta$ -Gal/*GenomONE-Neo*

(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).

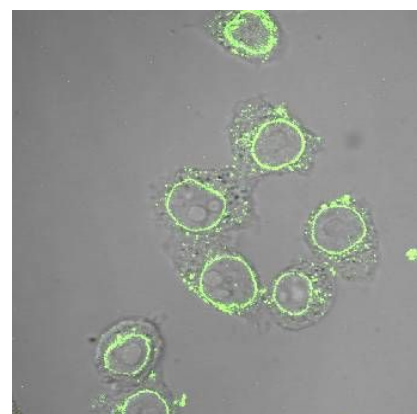
▶ **Antibody delivery into living cells using *GenomONE-CAb***



**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 (Efficiency of delivery of FITC-TCTP; 99%)
Y. Kondo	<i>Curr. Protoc. Immunol.</i> , Chapter 10, Unit 10.17D. 1-9 (2010)	20376840	K562	RNaseT1
S. Kamitani	<i>J. Biol. Chem.</i> , < <i>in press</i> >	20534589	Swiss 3T3	PMT (Pasteurella multocida toxin)

### 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)	$\beta$ -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, Raw264.7	anti-NPC, anti- $\alpha$ tubulin, Mouse control IgG
Y. Kondo	<i>Curr. Protoc. Immunol.</i> , Chapter 2, Unit 2.16. 1-12 (2010)	20376843	Raw264.7, Monkey kidney (2-2 cell)	anti-NPC antibody, Flag-Cre
S. Balasubramanian	<i>PLoS One.</i> , 5(7), e11470 (2010).	20635003	adult feline cardiomyocytes	beta-actin antibody

## ► 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

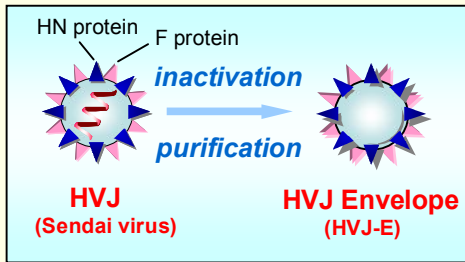
BHK21	Hamster kidney fibroblast	~100%
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### Rabbit IgG (Alexa488-labeled) delivery

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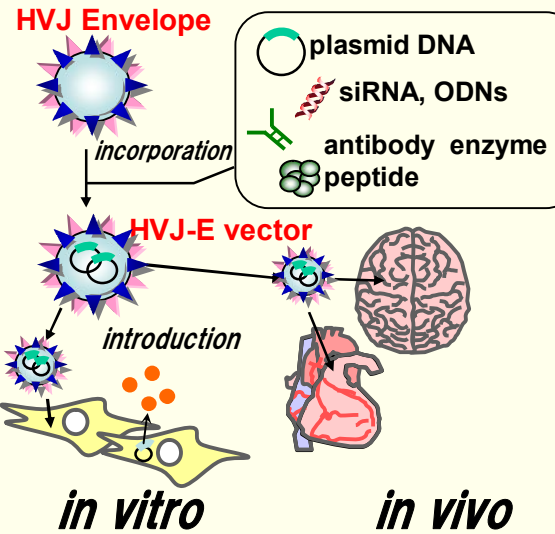
# What is HVJ Envelope (HVJ-E) ?

## What is HVJ Envelope (HVJ-E) ?



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.

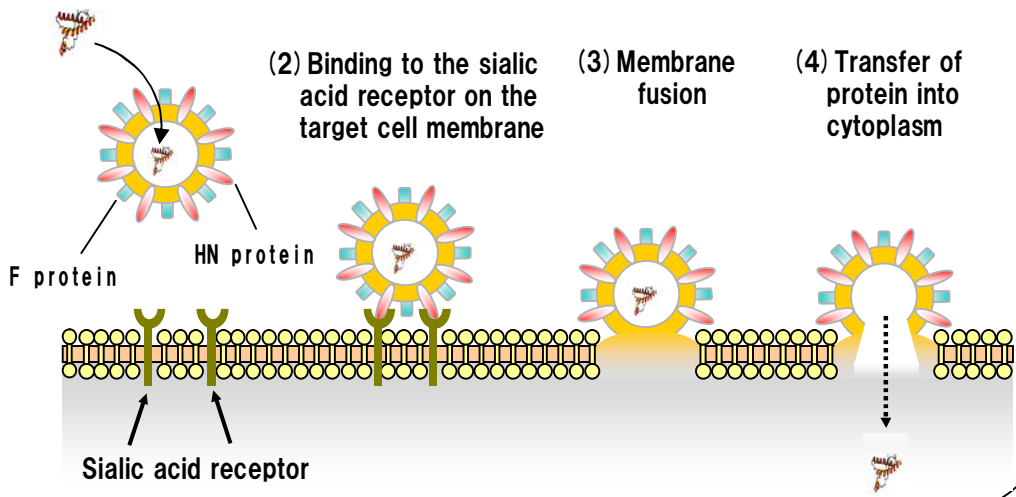
## Transfection using HVJ-E vector



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

(1) Protein is incorporated into HVJ-E

### Principle of protein introduction by HVJ-E



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