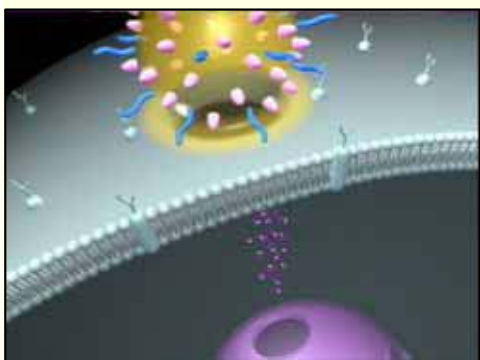


# GenomONE™

## Transfection and Cell Fusion Kits

For DNA, siRNA, Protein Delivery

**GenomONE™-Neo EX**

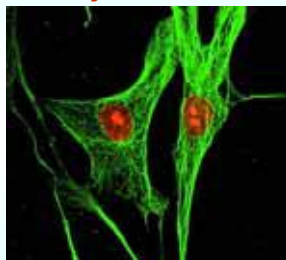


Allows **TRANSFECTION** of diverse molecules (DNA, siRNA, proteins etc.) *in vitro* and *in vivo* by means of **MEMBRANE FUSION**

For Antibody Delivery

**GenomONE™-CAB EX**

Delivery of anti- $\alpha$ -tubulin antibody into HS68 cells



nucleus of each cell was stained with SYTO82 (red)

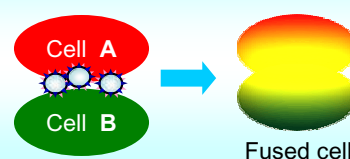
Optimized for **ANTIBODY DELIVERY** into **LIVING CELLS**

For Cell Fusion

**GenomONE™-CF EX**

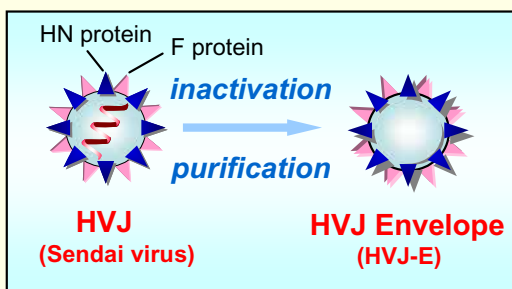
New **CELL FUSION REAGENT** which can replace PEG

Cell fusion triggered by HVJ-E



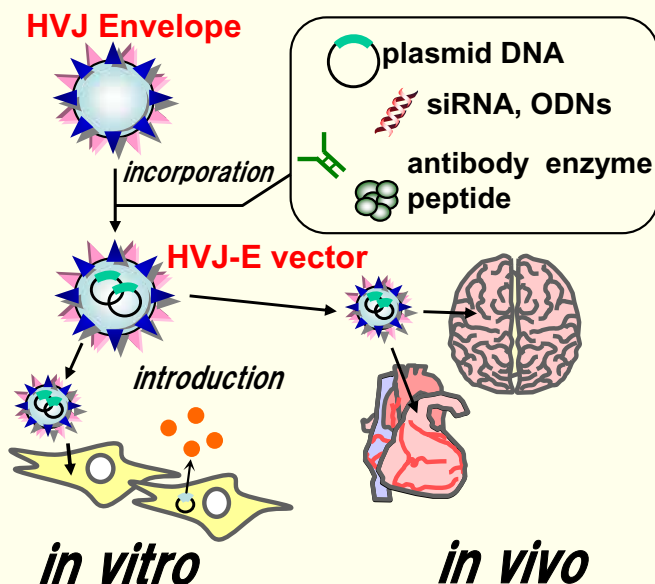
- Simple to use
- Much lower cytotoxicity than PEG
- Yield efficient hybridoma formation than PEG

### 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



**ISK** ISHIHARA SANGYO KAISHA, LTD.

Distributor



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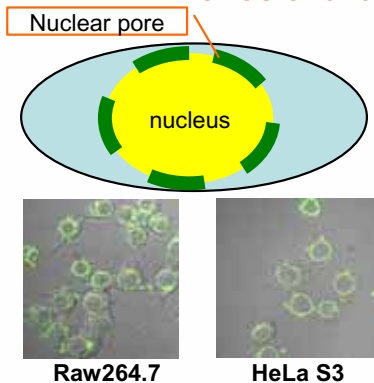
# For Antibody Delivery **GenomONE™-CAB EX**

## Antibody Delivery Reagent

- ◆ Analysis of intracellular function.
- ◆ Live cell imaging is performed.
- ◆ Screening of antibody neutralization.

**GenomONE™-CAB EX Antibody Delivery Reagent** is a next-generation tool for antibody introduction into cells. With this kit, antibody can be incorporated into the HVJ Envelope (HVJ-E), a transfection tool making use of the membrane fusing ability of inactivated Sendai virus (HVJ: Hemagglutinating Virus of Japan). If cells are treated with HVJ-E including antibody, it will be possible to achieve efficient introduction of IgG antibodies into the cytoplasm. This kit provides a totally new methodology for experiments, overcoming the difficulties involved in experiments using conventional lipid-based reagents by which antibodies are introduced into cells by means of endocytosis.

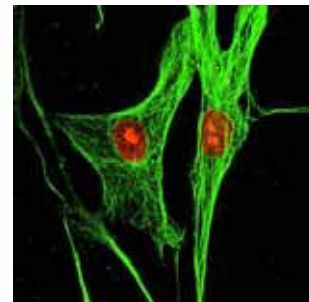
### ● Intracellular delivery of anti-NPC monoclonal antibody



The anti-NPC antibody, introduced into living cells, moves onto the nuclear membrane, holding its activity, where it binds specifically to the antigen (resulting in a ring-shaped chromatic response of the nuclear membrane)

【Antibody】  
Monoclonal Anti-NPC, Clone 414, Mouse IgG1

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



Specific binding to tubulin filaments is visible  
nucleus of each cell was stained with SYTO82 (red)

【Antibody】  
Monoclonal Anti-tubulin, Clone DM1A, Mouse IgG1

### ● Performance compared with existing reagents for protein introduction

Hs68 cells(anti-NPC antibody)observed by confocal laser scanning microscopy		
<b>GenomONE™- CAB</b>	<b>Reagent (A)</b>	<b>Reagent (B)</b>
Antibody 1 $\mu$ g/well	Antibody 1 $\mu$ g/well	Antibody 1 $\mu$ g/well

#### ▶ Kit components

		<b>ISK-AB001-EX</b>	<b>ISK-AB004-EX</b>
Freeze-dried HVJ-E	(0.26mL/tube)	1Vial	4Vials
Reagent I	(0.26mL/tube)	1Vial	4Vials
Reagent II	(0.3mL/tube)	1Vial	1Vial
Reagent III	(1mL/tube)	1Vial	4Vials
Buffer	(6.5mL/tube)	1Vial	1Vial
Instruction Manual		1	1

# For DNA, siRNA, Protein Delivery

## GenomONE™-Neo EX

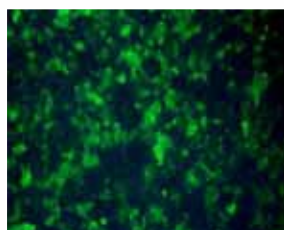
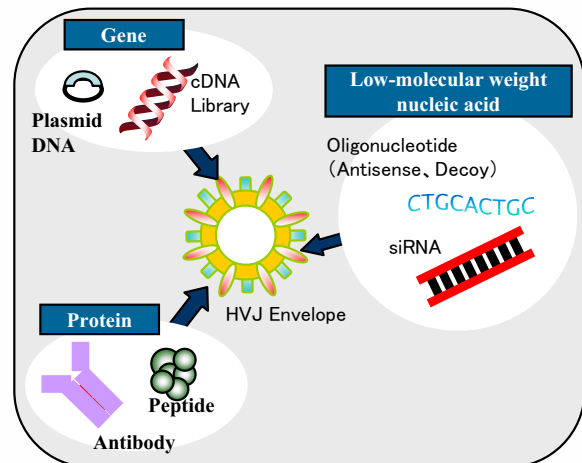
## HVJ Envelope Vector kit

- ◆ Unique, efficient transfection of siRNA, DNA, oligonucleotides, proteins.
- ◆ Low toxicity with primary cell lines.
- ◆ Protocols for in vivo transfection.

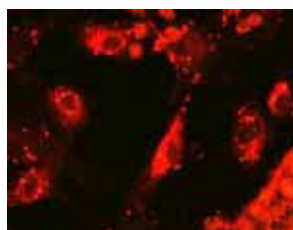
HVJ (Hemagglutinating Virus of Japan) Envelope VECTOR KIT is a tool for transfection of molecules (plasmid DNAs, siRNAs, oligonucleotides, proteins, antibodies etc.) into cells and animal tissue by means of membrane fusion. The HVJ envelope, carrying the molecule to be transfected, is composed of a completely inactivated and purified HVJ (Sendai virus) while preserving the cell membrane-fusing capability of the envelope.

### Allows transfection of diverse molecules

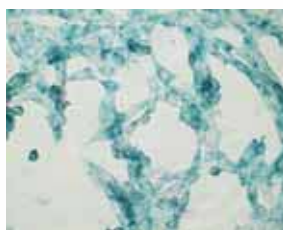
- Diverse molecules, ranging from genes (plasmid DNA, etc.) to oligonucleotides, siRNA, peptides and various proteins (antibodies, etc.), can be incorporated into the HVJ envelope for transfection.
- Direct transfection of molecules into cells by means of membrane fusion, without involving chemical modification, leads to high functional expression. This feature is particularly useful in analyzing the gene function by siRNA transfection.
- Multiple molecules can be incorporated into the envelope at the same time, allowing high throughput screening.



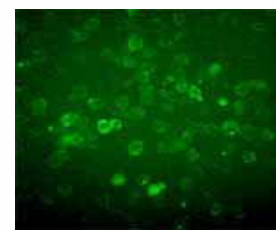
ODN introduction to 3T3-L1(differentiated)



siRNA introduction to rat primary cardiac myocytes

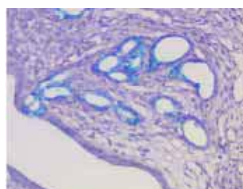


$\beta$ -galactosidase introduction to NIH3T3 (X-Gal staining)



Rabbit IgG introduction to HL-60

### Applicable to in vivo use



$\beta$ -galactosidase gene expression in mouse uterus (X-Gal staining)



$\beta$ -galactosidase gene expression in mouse lung (X-Gal staining)



$\beta$ -gal only



$\beta$ -gal+GenomONE

$\beta$ -galactosidase introduction into intradermally implanted Colon26 mouse tumor (X-Gal staining)

### Kit components

		ISK-GN001-EX	ISK-GN004-EX
Freeze-dried HVJ-E	(0.26mL/tube)	1Vial	4Vials
Reagent A	(0.5mL/tube)	1Vial	1Vial
Reagent B	(0.3mL/tube)	1Vial	1Vial
Reagent C	(1mL/tube)	1Vial	1Vial
Buffer	(6.5mL/tube)	1Vial	1Vial
Instruction Manual		1	1

# For Cell Fusion **GenomONE™-CF<sub>EX</sub>**

A new cell fusion reagent which can replace PEG

- ◆ Simple to use
- ◆ Much lower cytotoxicity than PEG
- ◆ Yields efficient hybridoma formation

Normal BALB/c mouse splenocytes ( $1 \times 10^8$  cells) not sensitized with antigen were fused to X63-Ag8.653 myeloma cells ( $1 \times 10^7$  cells) using **GenomONE™-CF<sub>EX</sub>** or PEG1500. One-fifth of the fused cell suspension obtained with each agent were inoculated onto a 96-well plate (Day 0). Beginning the following day, half of the culture medium (10%FBS/RPMI1640) was replaced with HAT medium at five points of time (Days 1, 2, 3, 5, and 8), and the growth of colonies in each well was assessed on Days 10 - 11 to determine the hybridoma-positive rate (an indicator of efficiency of fusion). On Day 12, mouse antibody level (IgG + IgA + IgM) in the supernatant was measured by ELISA, to calculate the antibody production-positive rate. The effect of adding a commercially available hybridoma supplement to the medium after fusion was also assessed (supplement was also added to the HAT medium).

## ● Hybridoma-positive rate (efficiency of cell fusion) and antibody (mouse Ig) production-positive rate

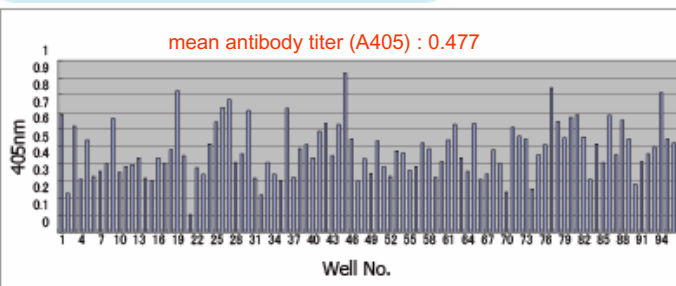
Use of **GenomONE™-CF<sub>EX</sub>** resulted in more efficient formation of antibody-producing hybridoma than that of PEG. The efficiency of cell fusion mediated by **GenomONE™-CF<sub>EX</sub>** was increased by the addition of hybridoma supplement to the medium used for incubation after cell fusion.

	Supplement	Hybridoma - positive rate	Antibody production - positive rate
<b>GenomONE-CF EX</b>	—	38/96 (40%)	9/96 (9%)
	+	96/96 (100%)	96/96 (100%)
PEG1500	—	3/96 (3%)	1/96 (1%)
	+	36/96 (38%)	9/96 (9%)

## ● Antibody production

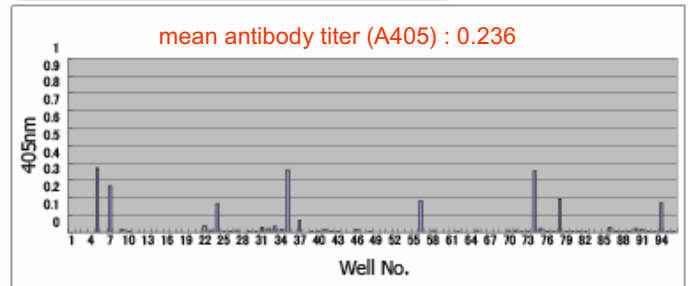
When the cells fused using **GenomONE™-CF<sub>EX</sub>** were incubated in medium containing a supplement, colony growth improved and the amount of antibody produced per antibody-positive well increased, resulting in a markedly greater mean amount of antibody produced, compared to the method of cell fusion using PEG.

### **GenomONE-CF EX** / with supplement



The vertical axis of this graph indicates the amount of antibody produced. The wells were considered antibody (mouse Ig) production-positive if A405 absorbance was  $\geq 0.05$ .

### PEG1500 / with supplement



(The results shown above are an example, and experimental conditions need to be optimized depending on the type of antigen, myeloma cell, supplement, etc. used)

### ➤ Kit components

Freeze-dried HVJ-E	(0.26mL/tube)	1Vial
HVJ-E suspending buffer	(0.5mL/tube)	1Vial
Cell fusion buffer (20X concentrate)	(10mL/tube)	1Vial
Instruction Manual		1

[http://www.cosmobio.co.jp/export\\_e/products/cells/products\\_ISK\\_20070518.asp](http://www.cosmobio.co.jp/export_e/products/cells/products_ISK_20070518.asp)

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