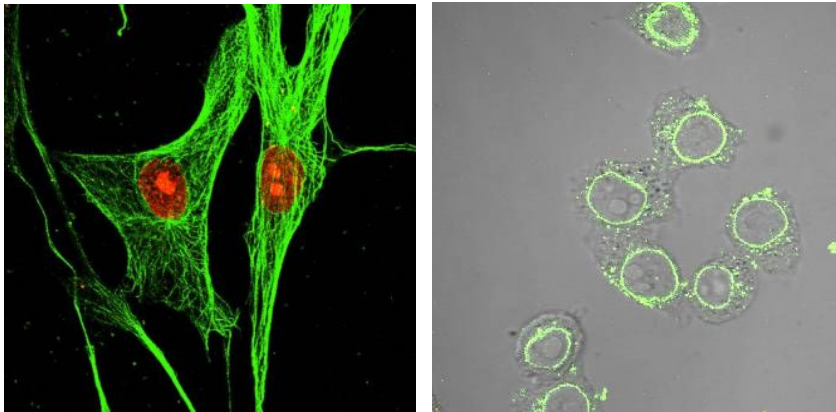


# ***GenomONE™-CAB EX***

## ***Antibody Delivery Reagent***

HVJ Envelope Vector (For Research Use)

# **Data Sheet**



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

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

# I . *GenomONE-CAb* Antibody Delivery Reagent

**This is a tool for efficient introduction of IgG antibodies into living cells using HVJ-E envelope (HVJ-E) vector for the purpose of analysis of the functions of cells and intracellular proteins.**

## [Introduction]

Because antibodies cannot enter cells, past experiments using antibodies focused primarily on extracellular molecules. If target molecules in living cells can be exposed to antibodies, it will be possible to pursue new dimensions of research related to cell function analysis, exploration of target molecules for disease diagnosis and treatment, and so on.

***GenomONE-CAb Antibody Delivery Reagent*** is a next-generation tool for antibody introduction into cells which can meet such needs.

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.

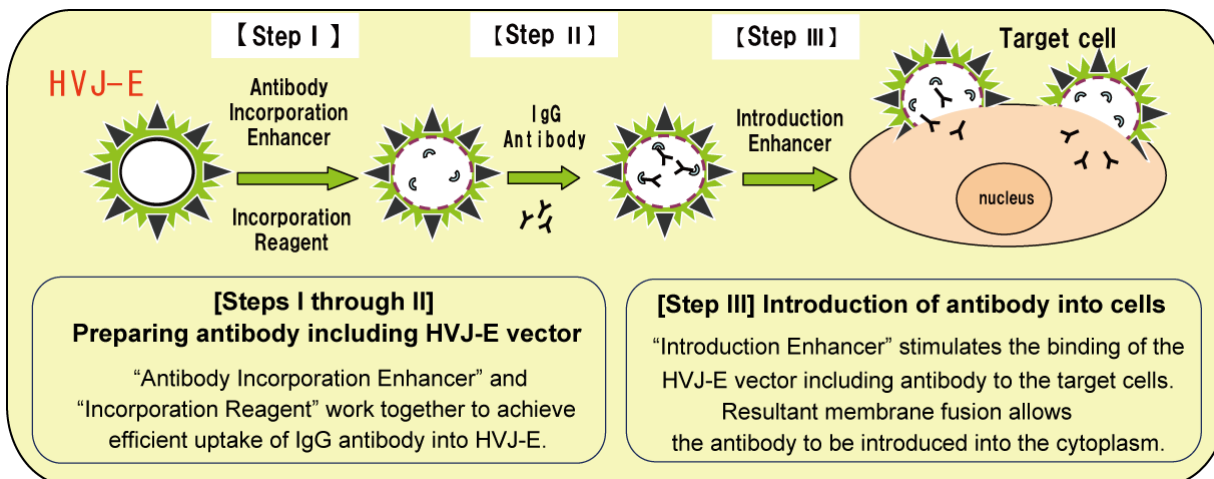
## [Principle of antibody introduction]

With this system, the IgG antibody incorporation enhancer\*, which was developed by our company, improves the efficiency of inclusion of IgG molecules into HVJ-E markedly compared to the existing HVJ-E vectors (***GenomONE-Neo EX***) marketed in 2007.

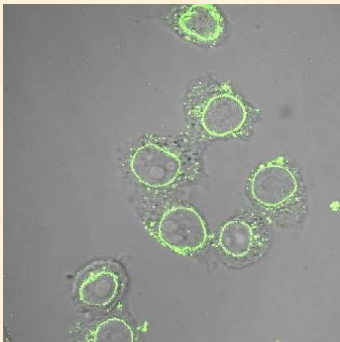
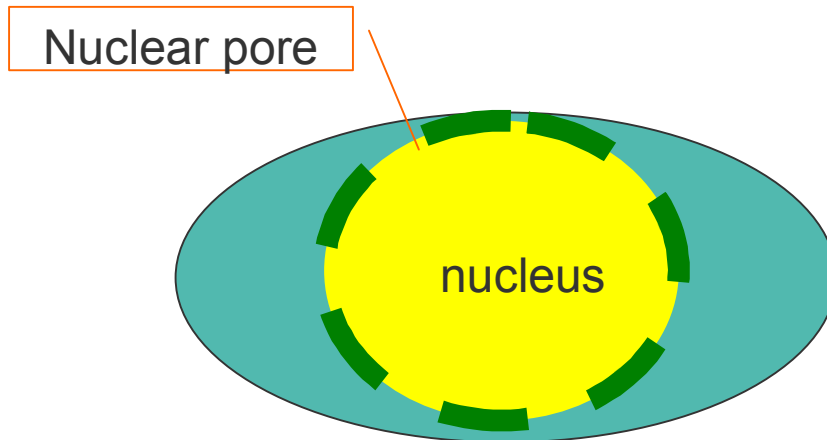
Thanks to this feature, this system allows efficient introduction of IgG molecules into the cytoplasm.

\* Papers related to ***GenomONE-CAb*** published by our company.

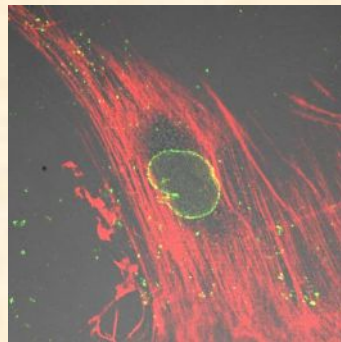
Kondo Y. *et al.*: Efficient delivery of antibody into living cells using a novel HVJ envelope vector system. *J. Immunol. Methods*, 332,10-17(2008).



## II. An example of antibody introduction into living cells (1) Intracellular delivery of anti-NPC monoclonal antibody

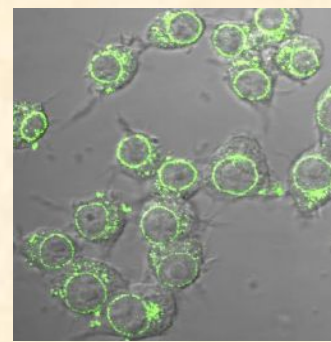


HeLa S3  
(ATCC-CCL 2.2)



Hs68  
(ATCC-CRL1635)

Actin filaments were stained  
with phalloidin (red)



Raw264.7  
(ATCC-TIB-71)

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)

F(ab')<sub>2</sub> fragment should be used as the fluorescence-labeled secondary antibody. If whole antibody is used, non-specific binding may take place, making it impossible for specific chromatic responses to appear.

### 【Protocol】

Cells, 1×10<sup>4</sup> cells/well 10%FCS/DME (8-well chambered coverglass: Nunc 155411)

↓ delivery of Anti-NPC mAb (2.5 μg) into cells using *GenomONE-CAb*

↓ incubation for 2 hr at 37°C

↓ wash the cells (PBS)

↓ fix the cells (4% PFA; for 15min, r.t.→PBS wash→0.2% Triton X-100; for 5min, r.t.  
→PBS wash→1%BSA/PBS; for 10min, r.t.)

↓ Alexa Fluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> Fragment (Invitrogen A11017)

↓ wash the cells (PBS)

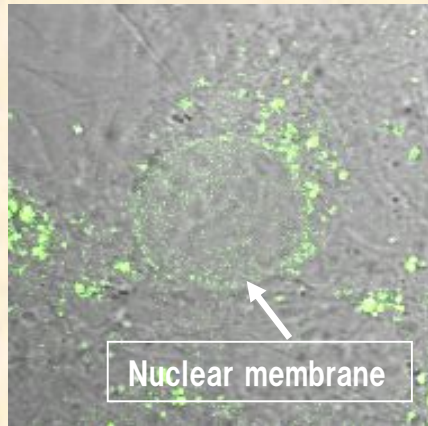
↓ observation by confocal laser scanning microscopy

【Antibody delivered into living cells】 Monoclonal Anti-Nuclear Pore Complex Proteins,  
Clone 414, Mouse IgG<sub>1</sub> (SIGMA, N8786)

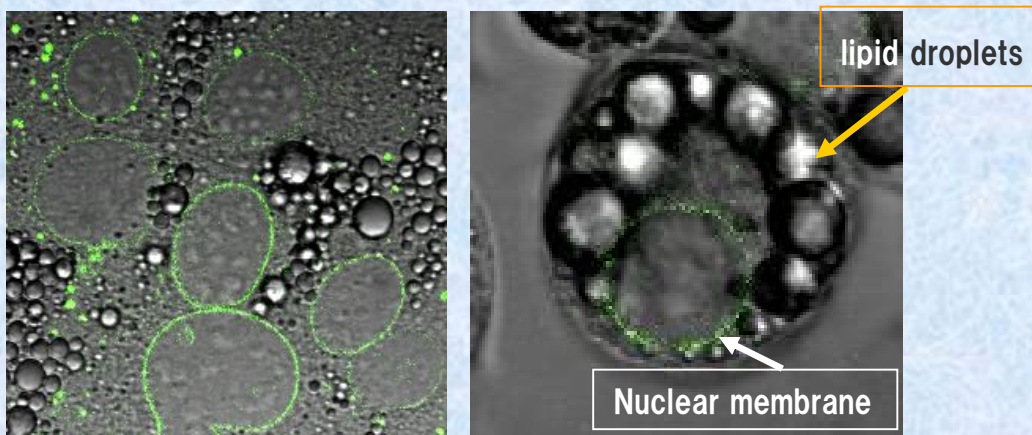
## II. An example of antibody introduction into living cells (2)

### Introduction of anti-NPC antibody into 3T3-L1 cells

(A) Undifferentiated 3T3-L1 cells



(B) Differentiated 3T3-L1 cells (adipocytes)



It has been demonstrated that anti-NPC antibody can specifically binds to antigen on nuclear membrane not only in undifferentiated cells (A) but also in cells which have differentiated into adipocytes (B)

#### 【Antibody delivered into living cells】

Monoclonal Anti-Nuclear Pore Complex Proteins, Clone 414, Mouse IgG1 (SIGMA, N8786)

#### 【Protocol】

Seed 3T3-L1 mouse embryo fibroblast (ATCC-CL-173),  $1 \times 10^4$  cells/well/8-well chambered coverglass, 10%FCS-DMEM

↓ two days after seeding (confluent), add MDI (0.5mM Methylisobutylxanthine,  $1 \mu\text{M}$  Dexamethasone,  $10 \mu\text{g/mL}$  Insulin)

↓ two days after differentiation: medium change to 10%FCS-DMEM (+Insulin), change the medium every two days

↓ seven days after differentiation: delivery of mAb ( $2.5 \mu\text{g}$ ) into cells using *GenomONE-CAB*

↓ incubation for 4hr at  $37^\circ\text{C}$

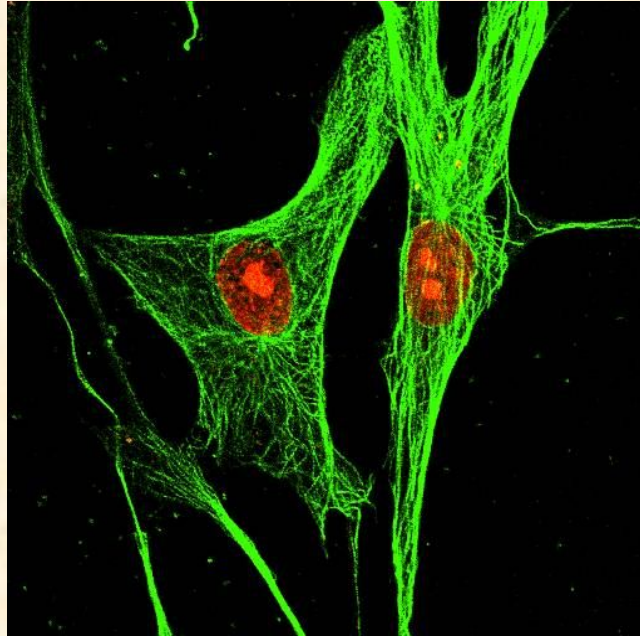
↓ fix the cells (4% PFA; for 15 min, r.t. → 0.2% Triton X-100; for 5 min, r.t.)

↓ Alexa Fluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> <Invitrogen A11017>,  $2 \mu\text{g/mL} \times 200 \mu\text{L}$ , incubation for 1hr at r.t.

↓ wash the cells (PBS)

observation by confocal laser scanning microscopy

## II. An example of antibody introduction into living cells (3) Introduction of anti- $\alpha$ -tubulin antibody into Hs68 cells



### Specific binding to tubulin filaments is visible

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

$F(ab')_2$  fragment should be used as the fluorescence-labeled secondary antibody. If whole antibody is used, non-specific binding may take place, making it impossible for specific chromatic responses to appear.

#### 【Antibody】

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

#### 【Protocol】

Hs68: Human foreskin fibroblast (ATCC-CRL-1635),  $1 \times 10^4$  cells/well,  
0.5%FCS/DMEM (8 well-chambered coverglass ; Nunc 155411)

↓ delivery of Anti- $\alpha$ -tubulin mAb (5  $\mu$ g) into cells using *GenomONE-CAb*

↓ incubation for 2hr at 37°C

↓ wash the cells (PBS)

↓ fix the cells (4% PFA: for 15min at r.t., 0.2% Triton X-100: for 5 min at r.t.,  
1%BSA: o/n)

↓ Alexa Fluor 488-Goat Anti-Mouse IgG,  $F(ab')_2$  <Invitrogen A11017>

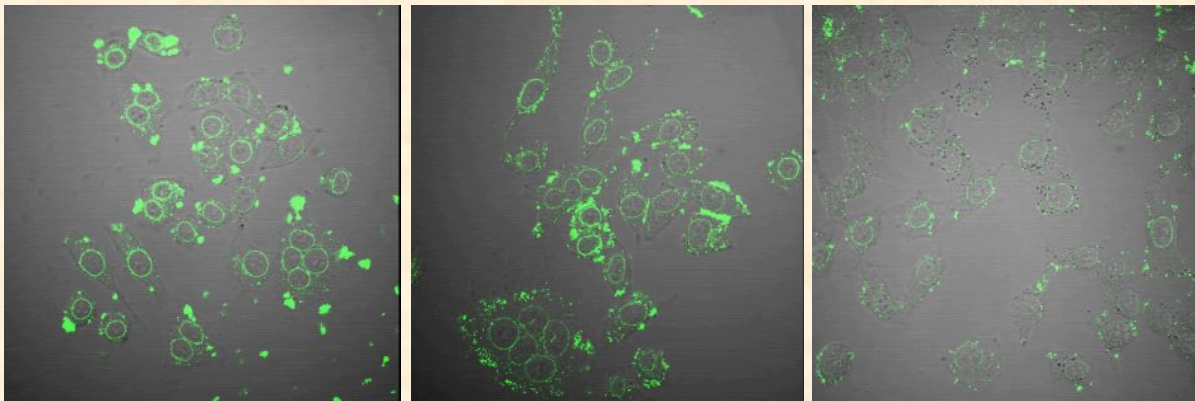
↓ wash the cells (PBS)

↓ SYTO 82 (Invitrogen S11363)

↓ observation by confocal laser scanning microscopy

### III. Stability of the delivered antibody

Delivery of anti-NPC (Nuclear Pore Complex Proteins) antibody into HeLa S3 cells



< 2hr >

< 24hr >

< 48hr >

Time after antibody introduction

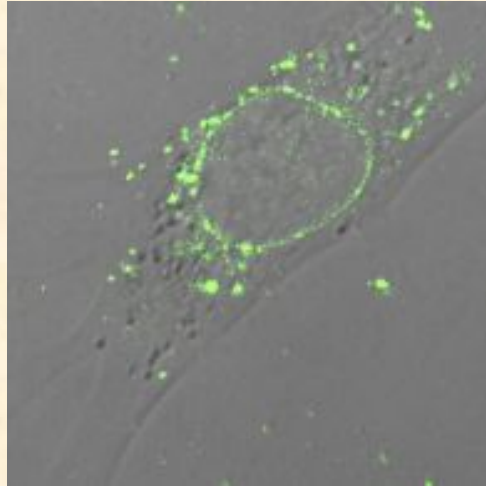
Similar fluorescence labeling images were obtained up to 24 hrs after delivery of anti-NPC antibody, suggesting that NPC was captured by antibodies within two hours after introduction and that antibody-antigen complexes were stable for at least 24 hrs.

【Cell line】 HeLa S3: Human, cervical epithelioid carcinoma (ATCC CCL 2.2)

【Antibody delivered into cells】 Monoclonal Anti-Nuclear Pore Complex Proteins, Clone 414, Mouse IgG<sub>1</sub> (SIGMA, N8786)

## IV. Introduction of fluorescence-labeled anti-NPC antibody Application for live cell imaging

### Hs68 cells



Antibody-labeling image of a live cell 2 hrs after delivery of AlexaFluor488-labeled-Anti-NPC antibody\* into Hs68 cells. Fluorescence was observed around the nuclear membrane.

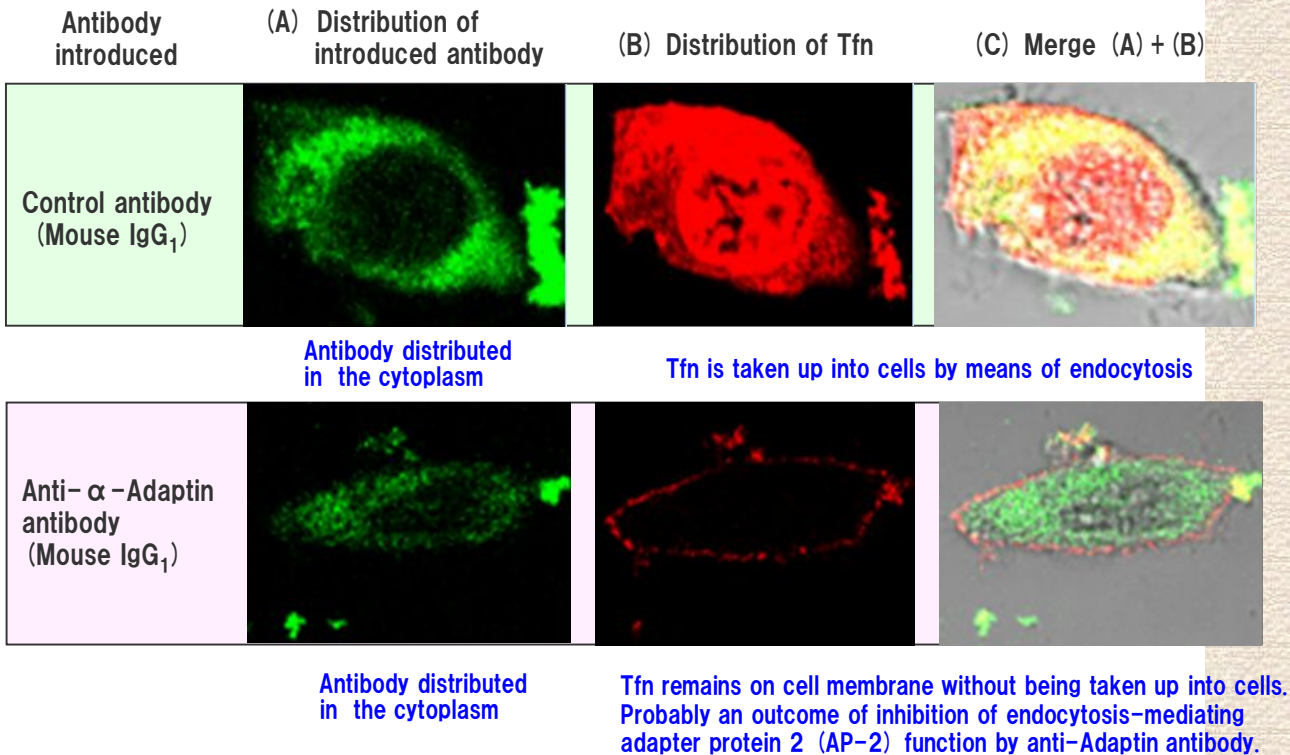
\* Prepared by using "Monoclonal Antibody Labeling Kit" (invitrogen, 20181)

**(Notice)**

If this kit is used for fluorescence-labeled antibodies or polyclonal antibodies (purified IgG), non-specific binding to cell surfaces may occur, possibly reducing the efficiency of antibody introduction into cells. In such cases, please refer to the trouble shooting in "Instruction Manual" and optimize the experimental condition.

# V. Functional analysis following intracellular antibody delivery ( 1 )

Endocytosis of transferrin (Tfn) is inhibited by delivery of anti- $\alpha$ -Adaptin antibody into HeLa S3  
(observed by confocal laser scanning microscopy)



## 【Protocol】

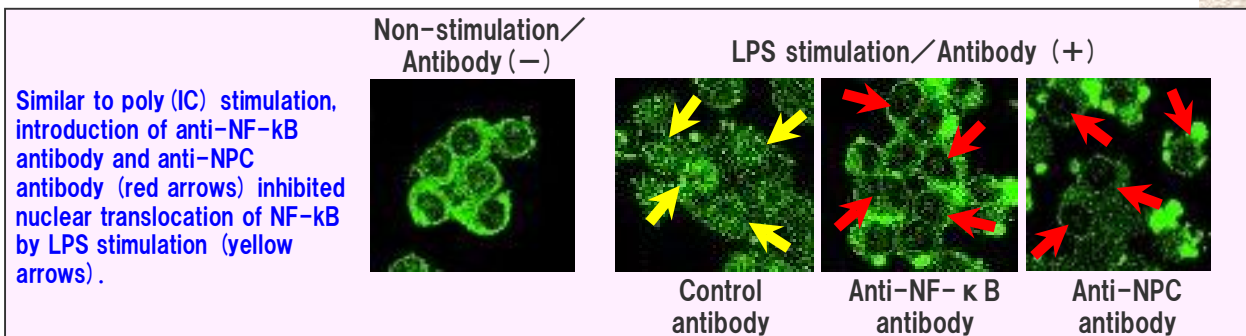
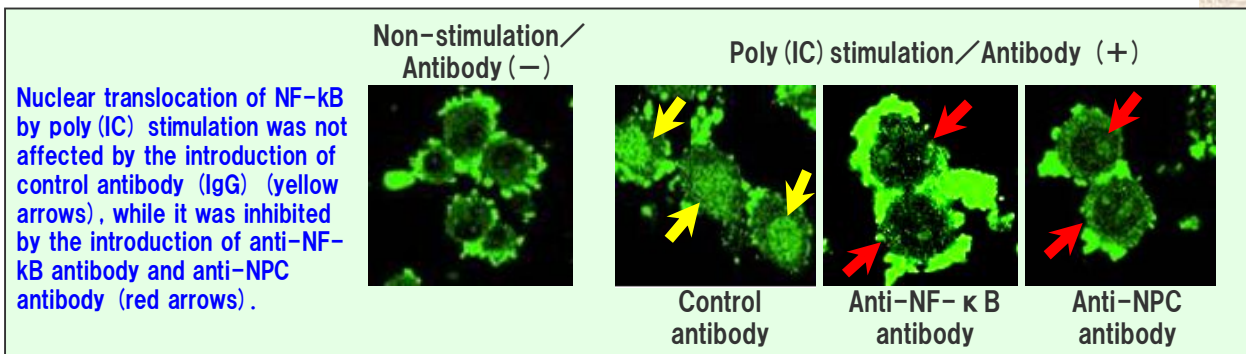
- HeLa S3 cells  $1 \times 10^4$  cells/well, 10%FBS-DMEM (8-well chambered coverglass: Nunc155411)
- ↓ delivery of 1  $\mu$ g of anti- $\alpha$ -Adaptin antibody (abcam, ab2730) or Control antibody (Sigma M9269) into cells using *GenomONE-CAb*
- ↓ incubation for 4 hr at 37°C
- ↓ renewal of medium with serum-free DMEM
- ↓ addition of AlexaFluor 594 conjugated transferrin (Invitrogen, T13343)
- ↓ incubation for 15 min 37°C
- ↓ wash the cells with cold 10%FBS-medium followed by cold PBS (-)
- ↓ fix the cells (4% PFA; for 15 min, r.t. → PBS wash → 0.2% Triton X-100; for 5 min, r.t. → PBS wash → 1%BSA/PBS; for 10 min, r.t.
- ↓ AlexaFluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> fragment, 1/500 dilution (Invitrogen, A11017)
- ↓ wash the cells with PBS (-) (3 times)
- ↓ observation by confocal laser scanning microscopy

# V. Functional analysis following intracellular antibody delivery (2)

## 【Experiment 1】

### Inhibition of nuclear translocation of NF- $\kappa$ B

Introduction of anti-NF- $\kappa$ B antibody or anti-NPC (nuclear pore complex protein) antibody to Raw 264.7 cells inhibited nuclear translocation of NF- $\kappa$ B (observed by confocal laser scanning microscopy).



#### 【Protocol / Experiment 1】

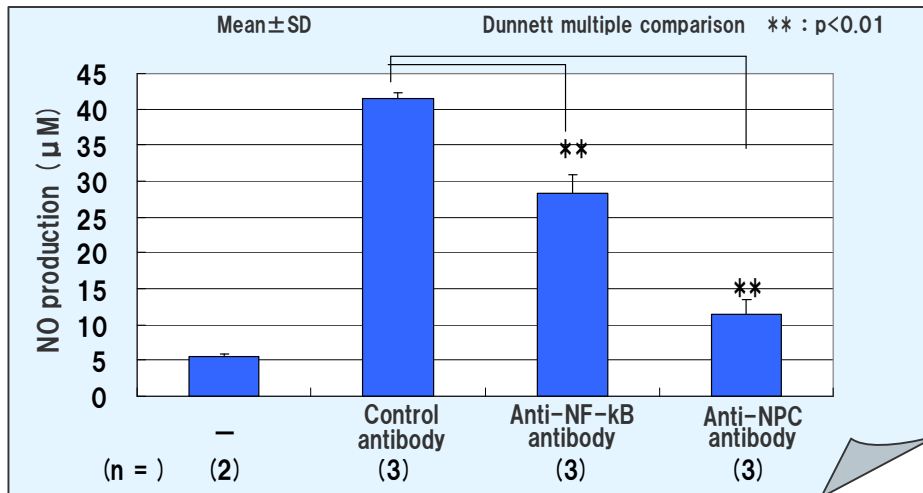
- Raw264.7 cells (ATCC TIB-71)  $1-3 \times 10^4$  cells/well, 10%FBS-DMEM (8-well chambered coverglass: Nunc155411)
- ↓ delivery of 0.8-1  $\mu$ g/well of Control antibody (Sigma, M9269), anti-NF- $\kappa$ B antibody (Santa Cruz Biotechnology Inc., Sc-8008) or anti-NPC antibody into cells using *GenomONE-Cab*
- ↓ incubation for 2-3 hr at 37°C
- ↓ addition of Poly (IC) (final 100  $\mu$ g/mL) or LPS (10  $\mu$ g/mL), 200  $\mu$ L/well
- ↓ incubation for 30 min 37°C (15 min for LPS-treated group)
- ↓ wash the cells with cold 10%FBS-DMEM followed by cold PBS (-)
- ↓ fix the cells (4% PFA; for 15 min, r.t. → PBS wash → 0.2% Triton X-100; for 5 min, r.t. → PBS wash → 3% BSA/PBS; for 10 min, r.t.)
- ↓ Rabbit anti-NF- $\kappa$ B p65 (c) antibody (kindly provided by Immuno-Biological Laboratories Co., Ltd., #8883); 0.4-4  $\mu$ g/mL, 3% BSA solution
- ↓ incubation for 1 hr at r.t.
- ↓ wash the cells with PBS (-) (3 times)
- ↓ AlexaFluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> fragment, 1/500 dilution (Invitrogen, A11017)
- ↓ wash the cells with PBS (-) (3 times)
- ↓ observation by confocal laser scanning microscopy

(This experiment was performed as joint research with Immuno-Biological Laboratories Co., Ltd. We express our heartfelt thanks to Dr. Kiyoshi Ishikawa and the other individuals involved.)

# V. Functional analysis following intracellular antibody delivery (3)

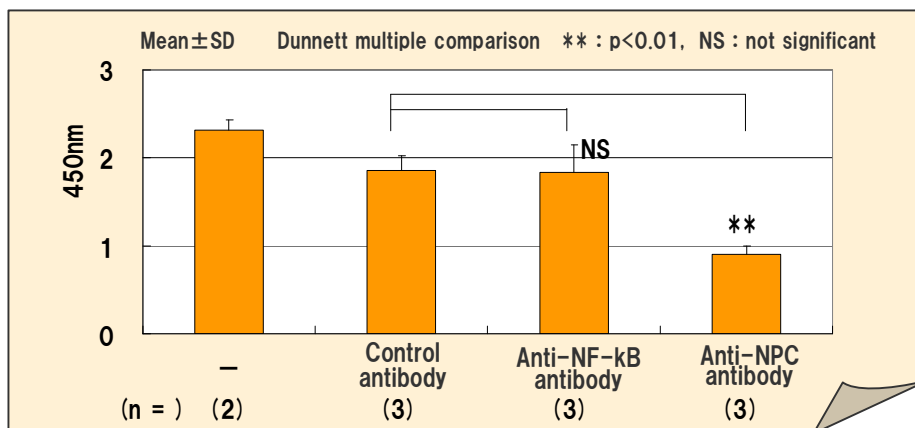
## 【Experiment 2】 Inhibition of Nitric Oxide production

Introduction of anti-NF- $\kappa$ B antibodies or anti-NPC (nuclear pore complex protein) antibodies to Raw 264.7 cells led to significant inhibition of nitric oxide (NO) production.



## 【Experiment 3】 Inhibition of cell growth

Introduction of anti-NPC antibody to Raw264.7 cells resulted in significant inhibition of cell growth. On the other hand, introduction of anti-NF- $\kappa$ B antibody did not produce inhibition.



### 【Protocol / Experiment 2 & 3】

Raw264.7 cells (ATCC TIB-71)  $4 \times 10^4$  cells/well, 10%FBS-DMEM (96-well plate: IWAKI 3860-096), overnight incubation  
 ↓ delivery of 0.8-1  $\mu$ g/well of Control antibody (Sigma, M9269), anti-NF- $\kappa$ B antibody (Santa Cruz Biotechnology Inc., Sc-8008) or anti-NPC antibody into cells using *GenomONE-CAB*

↓ incubation for 2-3 hr at 37°C

#### ↓【Exp.2】

↓ addition of LPS (10  $\mu$ g/mL), 200  $\mu$ L/well

↓ incubation for 19 hr at 37°C

↓ supernatant is added to each well of another 96-well plate: 150  $\mu$ L/well

↓ Griess Reagent ( $\times 2$  conc.), 50  $\mu$ L/well

↓ Left to stand for 10 min at r.t.

↓ A540nm

#### →【Exp.3】

↓ addition of Cell Count Reagent SF (nacalai tesque: 07553-44)

20  $\mu$ L/well

↓ incubation for 2-3 hr at 37°C

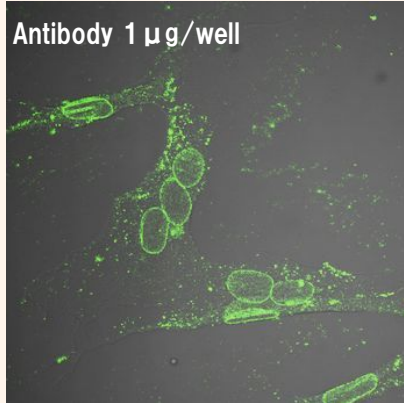
↓ 450nm

# VI. Performance compared with existing reagents for protein introduction (1)

Hs68 cells (anti-NPC antibody) / observed by confocal laser scanning microscopy

## *GenomONE-Cab*

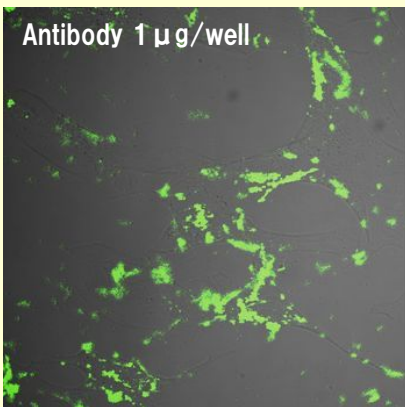
Antibody 1  $\mu$ g/well



Anti-NPC antibody, introduced into living cells, binds specifically to the antigen on the nuclear membrane.

## Reagent "P"

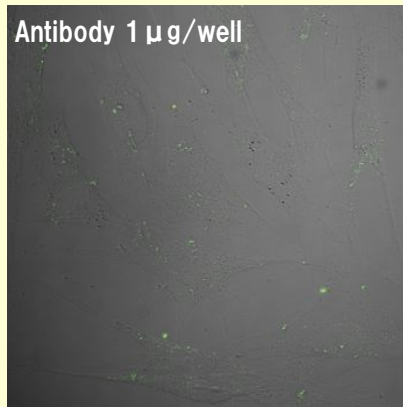
Antibody 1  $\mu$ g/well



Non-specific binding surrounding the cell surface was observed.

## Reagent "B"

Antibody 1  $\mu$ g/well



Specific binding to the antigen on the nuclear membrane was hardly observed.

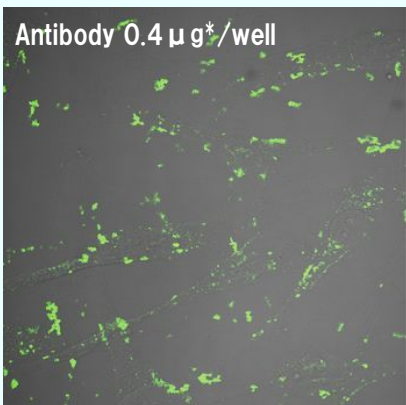
## Reagent "C"

Antibody 1  $\mu$ g/well

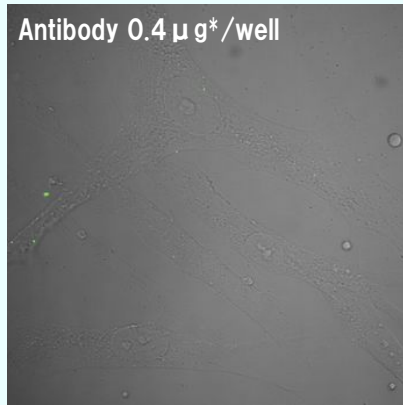


Specific binding to the antigen on the nuclear membrane was hardly observed.

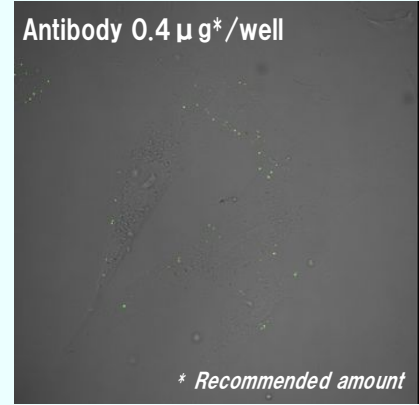
Antibody 0.4  $\mu$ g\*/well



Antibody 0.4  $\mu$ g\*/well



Antibody 0.4  $\mu$ g\*/well



\* Recommended amount

【Cells】 Hs68: Human, foreskin fibroblast (ATCC-CRL-1635),  $5 \times 10^3$  cells/well  
 【Antibody delivered into cells】 Monoclonal Anti-Nuclear Pore Complex Proteins, Clone 414,  
 Mouse IgG<sub>1</sub> (SIGMA, N8786)

## VI. Performance compared with existing reagents for protein introduction (2)

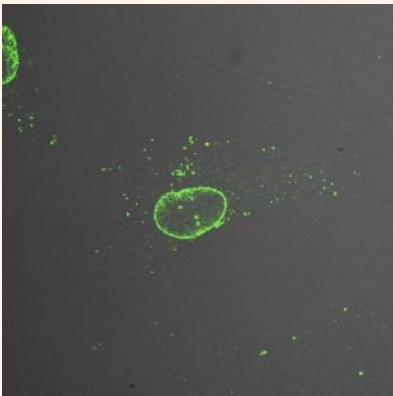
Hs68 cells (anti-NPC antibody) / observed by confocal laser scanning microscopy

### ***GenomONE-CAB***

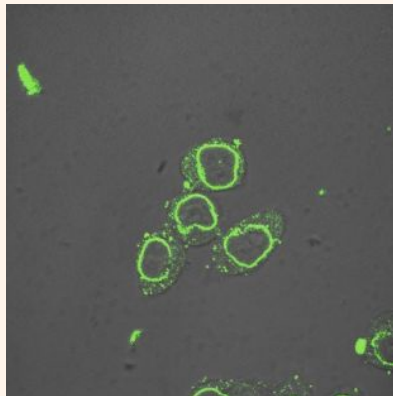
Anti-NPC antibody, introduced into living cells, binds specifically to the antigen on the nuclear membrane.

Antibody 1.1  $\mu$ g/well

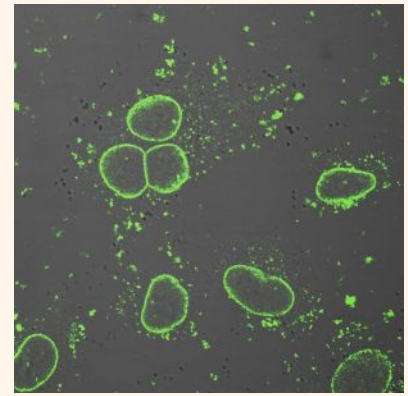
Hs68



HeLa S3



A549

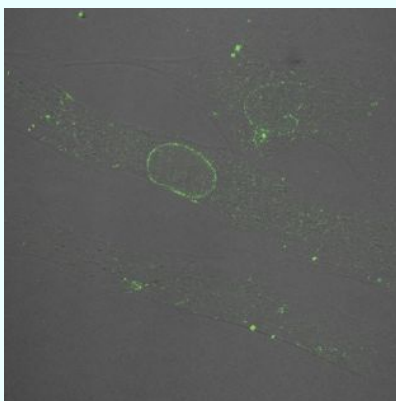


### **Antibody Delivery Reagent "DI"**

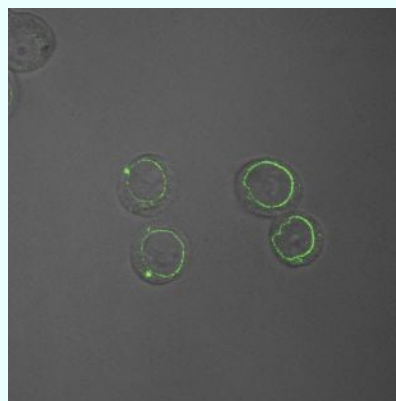
Efficiency of antibody delivery is relatively low as compared to *GenomONE-CAB*.

Antibody 1.0  $\mu$ g/well

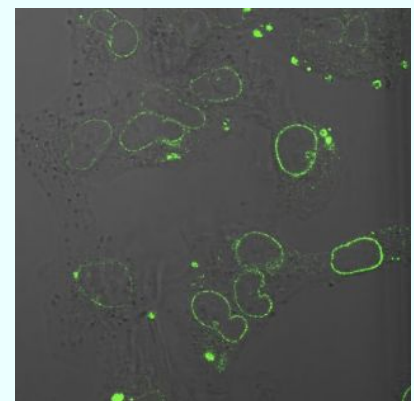
Hs68



HeLa S3



A549



【Cells】 Hs68: Human, foreskin fibroblast (ATCC CRL-1635),  $5 \times 10^3$  cells/well  
HeLa S3: Human, cervical epithelioid carcinoma (ATCC CCL 2.2),  $2 \times 10^4$  cells/well  
A549: Human, lung carcinoma (ATCC CCL-185),  $2 \times 10^4$  cells/well

【Antibody】

1<sup>st</sup> (delivered into living cells): Monoclonal Anti-Nuclear Pore Complex Proteins,  
Clone 414, Mouse IgG<sub>1</sub> (SIGMA, N8786)

2<sup>nd</sup>: Alexa Fluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> (Invitrogen, A11017)

## VII. Types of antibodies to which this kit is applicable

Delivery of various IgGs into HS-68 cells (Human foreskin fibroblast: ATCC CRL-1635)

Species	IgG subtype* <sup>1</sup>	Incorporation efficiency of antibody into HVJ-E* <sup>2</sup>	Introduction efficiency of antibody into HS-68 cells* <sup>3</sup>
Mouse	pAb	◎	△
	IgG1	◎	◎
	IgG2a	○	○
	IgG2b	◎	△
	IgG3	○	△
Rat	pAb	△	△
	IgG1	◎	○
	IgG2a	△	△
	IgG2b	○	○
	IgG2c	△	△
Human	IgG1	◎	○
	IgG2	◎	○
	IgG3	△	△
	IgG4	◎	○
Rabbit	pAb	○	○
Goat	pAb	△	△

\*<sup>1</sup>: pAb: polyclonal antibody, others: monoclonal antibody (control IgG)

\*<sup>2</sup>: Incorporation efficiency of antibody into HVJ-E:

◎: more than 40%, ○: 20-40%, △: less than 20%

\*<sup>3</sup>: Introduction of antibody into HS-68 cells:

◎: easy to introduce, ○: possible to introduce, △: not efficient but possible to introduce

(Notice)

If this kit is used for polyclonal antibodies (purified IgG) or fluorescence-labeled antibodies, non-specific binding to cell surfaces may occur, possibly reducing the efficiency of antibody introduction into cells. In such cases, refer to the trouble shooting in "Instruction Manual".

## VIII. Examples of cells into which IgG antibody can be introduced with this kit

Origin	Cell Line	Cell Type	Antibody Type
<b>Cancer, Adherent</b>			
Human	HeLa S3	cervical epithelioid carcinoma	mouse IgG pAb mouse IgG1 mAb
Human	A549	lung carcinoma	mouse IgG1 mAb
Human	SAS	tongue carcinoma	mouse IgG pAb
Human	HT1080	fibrosarcoma	mouse IgG pAb
Human	MCF-7	mammary carcinoma	mouse IgG2a mAb
Mouse	P19	embryonal carcinoma	mouse IgG pAb
Mouse	Raw264.7	leukemic monocyte	mouse IgG pAb
<b>Cancer, Non-adherent</b>			
Human	Jurkat	T cell leukemia	mouse IgG1 mAb
Mouse	P3-X63-Ag8.653	myeloma	mouse IgG1 mAb
<b>Normal, Adherent</b>			
Human	WI-38	lung fibroblast	mouse IgG1 mAb
Human	Hs68	skin fibroblast	mouse IgG1 mAb
Mouse	3T3-L1	embryo fibroblast, undifferentiated	mouse IgG1 mAb
Mouse	3T3-L1, adipocyte	embryo fibroblast, differentiated	mouse IgG1 mAb
Mouse	BNL CL2	embryo hepatocyte	mouse IgG1 mAb
Hamster	BHK-21	kidney fibroblast	mouse IgG1 mAb
<b>Normal, Primary cultured</b>			
Human	HAEC	aortic endothelial	mouse IgG1 mAb

pAb: polyclonal antibody, mAb: monoclonal antibody

### Reference

Kondo, Y. *et al.*: Efficient delivery of antibody into living cells using a novel HVJ envelope vector system. *J. Immunol. Methods*, 332,10-17(2008).

Kondo, Y. *et al.*: Efficient delivery of antibody into living cells using hemagglutinating virus of Japan (HVJ) envelope. *Curr. Protoc. Immunol.*, Chapter 2, Unit 2.16, 1-12 (2010).

Balasubramanian, S. *et al.*: Hypertrophic stimulation increases beta-actin dynamics in adult feline cardiomyocytes. *PLoS One.*, 5(7), e11470 (2010).

## IX. Application of *GenomONE-CAb* Antibody Delivery Reagent

***GenomONE-CAb* Antibody Delivery Reagent enables these experiments, for example:**

### ▶ **Analysis of intracellular function**

- An antibody is introduced into living cells to examine the distribution of the target molecule within them
- An antibody is introduced into living cells to suppress and clarify the function of target molecules
- Live cell imaging is performed

### ▶ **Screening of antibodies reacting to intracellular antigens**

- Antibodies binding to intact antigens in living cells and showing neutralization activity are screened for

### ▶ **Application to testing, diagnosis, and treatment**

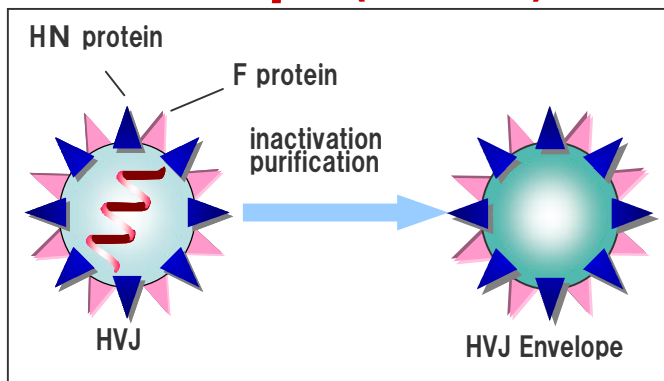
- New agents for testing and diagnosis are developed using antibodies capable of detecting target molecules in living cells
- Next-generation antibody-based drugs are created, which exert therapeutic effects through acting on intracellular target molecules

### ▶ **Advantages of intracellular antibody introduction (differences from existing knockout method)**

- Unlike post-transcriptional gene silencing (RNAi method, *etc.*), this method is expected to achieve specific inhibition by recognizing protein-protein interactions or post-translational modifications (addition of sugar chains, *etc.*)
- Nonspecific reactions (off-target effects of RNAi method, *etc.*) are unlikely to occur
- Unlike gene transfer and expression methods, introduction of antibody in amounts sufficient to exert efficacy can be achieved rapidly and simply, and this method is applicable to a wider range of types of experiments

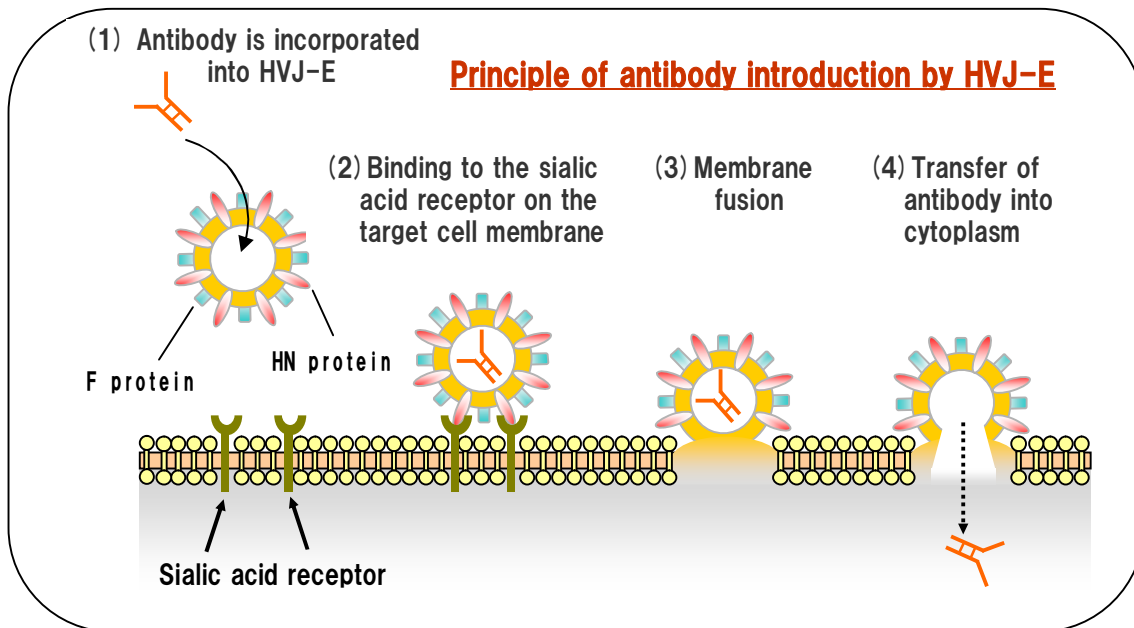
# X. Appendix

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



HVJ Envelope 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 of Sendai virus is retained. The genomic RNA of the Sendai virus contained in HVJ-E has been inactivated completely and has neither infective nor proliferative potentials in humans or experimental animals. HVJ-E can be used safely at ordinary laboratories, without requiring any special operations or facilities.

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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