HVJ Envelope Antibody Delivery Reagent

GenomONE™-CAb EX

Instruction Manual

(Ver. 2.1)

Precautions for use 2

1. Delivery of antibodies into living cells using HVJ-Envelope (HVJ-E)...................................3

Introduction..3
Principle of antibody delivery 3
Types of antibodies to which this kit is applicable..3
Advantages of this kit..4
Important precautions 4

2. Outline of the kit..4

Specification 4
Role of each reagent..4
Frequency of use 5
Storage, stability, and quality assurance 5
Quality 5

3. Procedure for incorporation of antibody into HVJ-E [Recommended protocol]…… …...6

Adherent cells..6
Suspension cells..8

4. Manipulations for related experiments ……………………………………… ………………..10

Methods for observation of the distribution (localization) of antibody introduced into viable cells……………………………………………………………………………………………..11

5. Precautions and trouble shooting 11

6. Types of antibodies to which this kit is applicable..12

7. Advantages of intracellular antibody introduction and possible applications 13

! This product is for use in laboratory research. It has not been approved for *in vitro* or *in vivo* use for the diagnosis or treatment of a patient and the seller advises against any such use.

! This package insert describes standard methods to be used with *GenomONE*™*-CAb EX* for delivery of IgG antibody. The methods described here yield reasonable efficiency of antibody delivery, though optimal conditions of delivery can vary depending on cell type. It is advisable to optimize the conditions of delivery, referring to the precautions listed in this package insert.

Precautions for use

1. **This product is sold for research purpose only. It may not be used for treatment or other clinical purposes or for intra- and extracorporeal diagnosis in humans or animals.**

2. When using this product for recombinant DNA experiments, rules for recombinant DNA experiments (stipulated in relevant statutes in the country of use or set forth by the safety committee of the facility concerned) must be followed, and experiments should only be carried out in laboratories properly equipped with facilities appropriate for recombinant DNA experiments.

3. Experiments using this product must only be carried out by investigators who have been trained in laboratory techniques and have knowledge of and skill in cell culture and genetic engineering.

4. Laboratory staff members working in the area where HVJ-E experiments are occurring should be informed of the properties of HVJ-E, in order to prevent accidents arising from inappropriate handling of it.

5. Although the HVJ (Sendai virus) contained in the HVJ envelope (HVJ-E) of this kit has been inactivated to completely eliminate its proliferative and infective potential, it retains membrane-fusion activity. Therefore, to prevent inhalation, attachment, unintended swallowing, or spread to eyes or nose of the HVJ-E particles, the product must be manipulated within a safety cabinet, wearing appropriate clothing (laboratory overalls) and protective items (plastic or latex gloves, mask, protective eyeglasses, etc.).

6. Do not pipette HVJ-E by mouth. Avoid splashing or generation of aerosols. Avoid contact of skin or mucous membranes with HVJ-E and other kit reagents. In the case of contact with skin or eyes, wash immediately with water. Membrane-fusion activity of HVJ-E is inactivated by autoclaving or treatment with detergent or 70% ethanol.

7. Empty containers of HVJ-E and tools and devices exposed to HVJ-E (pipettes, dishes, chips, etc.) must be handled carefully and disposed of after being autoclaved.

8. Although none of the other reagents contained in the kit is a toxic or powerful substance, they should be handled with protective items (laboratory overalls, gloves, mask, etc.).

9. The HVJ-E suspension has been confirmed by sterility testing to be free of contamination by bacteria or fungi. However, absence of contamination by all microorganisms cannot be guaranteed and appropriate procedures must be followed when using this product.

10. Freeze-dried HVJ-E and the reconstituted suspension should be stored at 2-8°C. Do not use HVJ-E beyond expiration date on label.

11. The proper use of this product is described in the instructions given in this package insert. Manufacturer (Ishihara Sangyo Kaisha, Ltd.) and distributors are not liable for any accident or damage arising from the use of this product which is not in strict compliance with these instructions

12. This product and its use are covered by the claims of one or more patents (including patents pending) and licensed for research use only. It may not be used for any commercial or other purpose or resold after modification or the like without prior written approval from manufacturer (Ishihara Sangyo Kaisha, Ltd.).

1. Delivery of antibodies into living cells using HVJ-Envelope (HVJ-E)

【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.

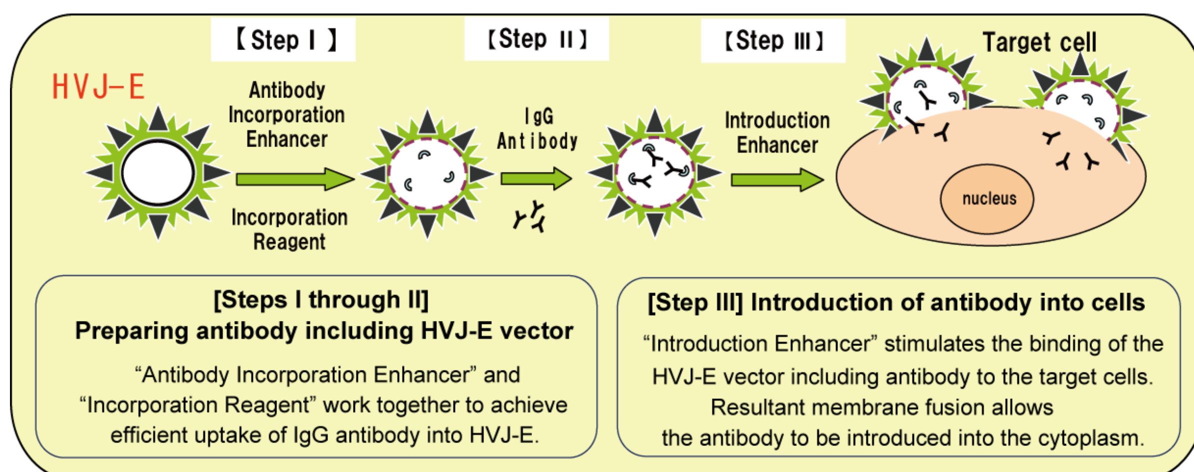
With this kit, antibody can be incorporated into the HVJ Envelope (HVJ-E), a transfection tool making use of the membrane fusing ability of inactivated Sendai virus (HVJ: Hemagglutinating Virus of Japan). If cells are treated with HVJ-E including antibody, it will be possible to achieve efficient introduction 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 delivery】

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*) .

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



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).

【Types of antibodies to which this kit is applicable】 Details, refer to Page 11

- Applicable to IgG antibodies（Mouse, Rat, Human, Rabbit, Goat）
- Especially, suitable for intracellular delivery of mouse IgG_1
- Antibody introduction efficiency will be lower if this kit is used for IgG_{2a}, IgG_{2b} and IgG_3 compared to IgG_1 antibodies
- Antibody introduction efficiency will be lower if this kit is used for IgM, IgA, IgE, IgY, single chain (scFv) or Fab antibodies compared to IgG antibodies
- Suitable for delivery of monoclonal antibody
- Relatively lower efficiency in case of polyclonal antibodies (purified IgG fractions) or antisera

【Advantages of this kit】

- The presence of 1%BSA, 0.1% gelatin or 0.1% NaN_3 (sodium azide) does not affect the efficiency of antibody incorporation into HVJ-E
- Serum in culture medium does not affect the efficiency of antibody introduction into cells

【Important precautions】

- If glycerol is contained in the antibody solution to be used for antibody incorporation, its concentration should not exceed 20%
- If this kit is used to introduce fluorescence-labeled antibody, nonspecific binding to cell surfaces may occur, possibly reducing the efficiency of antibody introduction into cells. Please refer to paragraph (2) on page 11 for troubleshooting
- $F(ab')_2$ fragment must be used as the labeled secondary antibody for fluorescent staining. If whole antibody is used, unspecific binding may occur, making it impossible for specific chromatic responses to appear. Please refer to paragraph (3) on page 11 for details and troubleshooting.

2. Outline of the kit

【Specification】 Storage:2−8℃

Cat. #	**HVJ-E (inactivated HVJ)** Freeze-dried 0.26 mL /vial (when reconstituted)	**Reagent Ⅰ** (antibody incorporation enhancer) Freeze-dried 0.26 mL/vial (when reconstituted)	**Reagent Ⅱ** (incorporation reagent) 0.3 mL/vial	**Reagent Ⅲ** (introduction enhancer) 1 mL/vial	**Buffer** (for suspension and dilution) 6.5 mL/vial
AB001EX	1 vial	1 vial	1 vial	1 vial	1 vial
AB004EX	4 vials	4 vials	1 vial	4 vials	1 vial

Storage:
Freeze-dried HVJ-E and Reagent Ⅰ : Moisture can reduce the activities.
Refrigerated at 2-8°C, sealed in an aluminum package. Keep dry.
Reagent Ⅱ, Ⅲ and Buffer : Refrigerated at 2-8°C.
Reconstituted HVJ-E and Reagent Ⅰ : see page 5.

【Role of each reagent】

- Freeze-dried HVJ-E: The main frame of the vector into which the molecule to be transferred is included. It fuses with the cell membrane, allowing the target molecule to be introduced into the cytoplasm.
- Reagent Ⅰ (antibody incorporation enhancer): A protein with high binding affinity with HVJ-E and IgG antibody which increases incorporation efficiency of antibody molecule into HVJ-E.
- Reagent Ⅱ (incorporation reagent): Increases permeability across the HVJ-E membrane and facilitate incorporation of antibody.
- Reagent Ⅲ (introduction enhancer): A positively-charged peptide which increases affinity between the IgG-bearing HVJ-E (HVJ-E vector) and the cell (or tissue) and thus increases the efficiency of delivery.
- Buffer: Neutral buffer of physiological concentration used for suspending or diluting HVJ-E or other purposes.

【Frequency of use】

- If used with the method described in this package insert, the product can be used for IgG antibody delivery as follows.

  With a 24-well plate
  AB-001EX (HVJ-E:1 vial): 25 reactions
  AB-004EX (HVJ-E:4 vials): 100 reactions

  With an 8-well chambered coverglass for fluorescence observation
  AB-001EX (HVJ-E:1 vial): 50 reactions
  AB-004EX (HVJ-E:4 vials): 200 reactions

【Storage, stability, and quality assurance】

- The period of guarantee of quality for “freeze-dried HVJ-E” and “freeze-dried Reagent Ⅰ” are printed on the aluminum package.
- Because the activity of “freeze-dried HVJ-E” and “freeze-dried Reagent Ⅰ” can be reduced by exposure to high temperature or high relative humidity, refrigerated storage with sealing in an aluminum package is required.
- Storage of reconstituted “HVJ-E suspension” and “Reagent Ⅰ solution” (prepared with “Buffer”)

  “HVJ-E suspension”
  Requires refrigerated storage (2-8°C) and should be used within 2 weeks.
  Since thawing of frozen suspension can reduce activity, the suspension should not be stored frozen.

  “Reagent Ⅰ solution”
  For continuous use, store in a refrigerator (2-8°C) for up to 2 weeks.
  For extended storage, freeze in working aliquots at -80°C for up to 3 months. Thawing after freezing is possible only once.

- We cannot guarantee the quality of HVJ-E after expiration of the period of guarantee of quality, product stored at temperatures other than those indicated in the instructions, or product subjected to superficial modification, drug treatment, or the like.

【Quality】

- Although HVJ-E uses HVJ (Sendai virus) as a raw material, the genomic RNA of HVJ has been completely inactivated by drug treatment*. The HVJ-E will not proliferate or exhibit pathogenic effects in humans or animals.

  *Reference: Prior, C. *et al.*: ***BioPharm***, 22-33 (Oct. 1996)
  Kaneda, Y. *et al.*: ***Advances in Genetics***, 53, 308-332 (2005).

> **!** HVJ-E retains membrane-fusion activity. Therefore, to prevent inhalation, attachment, unintended swallowing, or spread to eyes or nose of the HVJ-E particles, the product must be manipulated within a safety cabinet, wearing appropriate clothing (laboratory overalls) and protective items (plastic or latex gloves, mask, protective eyeglasses, etc.).

- Inactivation of HVJ has been confirmed for each lot by the viral proliferative potential rule-out test, using cultured cells and fertilized chicken eggs.
- Absence of contamination by bacteria and fungi has been confirmed by sterility testing.

> **!** Absence of contamination by all microorganisms cannot be guaranteed and appropriate procedures must be followed when using this product.

- Antibody can be delivered into living cells both in the presence and absence of serum.

3. Procedure for incorporation of antibody into HVJ-E [Recommended protocol]

（1）Adherent cells

■ **Recommended antibody level**：0.1～0.5 mg/mL

⚠ If glycerol is included as a stabilizer in the antibody solution, the solution needs to be diluted to a glycerol concentration below 20 (v/v)%, in order to avoid reduction of efficiency of antibody incorporation into HVJ-E or cell function. Efficiency of antibody inclusion will not be affected by the presence of 1% BSA or 0.1% gelatin in the antibody solution.

■ **Cell density**（For 24-well plate／cell densities for plates of other sizes are given on Page 7）:
Used for antibody introduction at 40～60% confluency. Seed the cells at 1～5×10^4 cells/0.5mL/well one day before introduction.

■ **Protocol**

[1] Preparation (reconstitution) of ”HVJ-E suspension” and “Reagent Ⅰ solution”
Ice-cooled buffer (0.26 mL) is added to each tube containing “freeze-dried HVJ-E” or “freeze-dried Reagent Ⅰ”. The mixture is pipetted gently, with care taken to avoid bubbling. A homogeneous suspension is thus prepared and stored refrigerated. Immediately before use, HVJ-E suspension, other reagents, and tubes should be cooled adequately in an ice bath. Storage conditions are given on page 5.

[2] Antibody incorporation into HVJ-E and introduction into cells （For the use of 24-well plates）

		Step	Amount of reagent
Incorporation	(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 10 μL
	(2)	Combination with “Reagent Ⅰ solution“ (Antibody Incorporation Enhancer) and agitation (tapping).	Reagent Ⅰ :10 μL
	(3)	Combination with “Reagent Ⅱ“ (Incorporation Reagent) and agitation[1] (tapping).	Reagent Ⅱ : 2 μL
	(4)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(5)	Combination with IgG antibody solution (0.1-0.5 mg/mL) and agitation (pipetted 20-30 times).	IgG solution: 10 μL (1-5 μg)
	(6)	Left to stand on ice for 5 minutes.	
	(7)*	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(8)*	Sediment suspended in the “Buffer” (pipetted 20-30 times).	Buffer: 10 μL
	(9)	Combination with “Reagent Ⅲ”(Introduction Enhancer) and agitation (tapping)	Reagent Ⅲ: 12.5 μL
	(10)	HVJ-E vector suspension is combined with the cell culture in a well and incubated at 37°C under 5%CO_2 (medium renewed as needed)[2]. Serum in the medium does not affect the efficiency of antibody delivery into cells.	Suspension ((8) + (9)): 22.5 μL (for one well)
	(11)	Incubated at 37°C under 5%CO_2.	

- Steps (1) through (9) should be performed on ice.
- * Steps (7) and (8) may be skipped if the additive to the antibody solution affects neither cell viability nor cell function.

[1] The amount of “Reagent Ⅱ“ added should equal to 1/10 of the fluid volume before addition (Steps (1)+(2)).
[2] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (8) + (9)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 2 hours of exposure.

➢ Amount of reagent for each plate size

Plate size	Incorporation Step				Introduction Step		
	HVJ-E (1)	Reagent Ⅰ (2)	Reagent Ⅱ (3)	Antibody (5)	Buffer (8)	Reagent Ⅲ (9)	Amount of HVJ-E vector to be treated (10)
6-well	40μL	40μL	8μL	40μL	40μL	50μL	90μL×1well
24-well	10μL	10μL	2μL	10μL	10μL	12.5μL	22.5μL×1well
96-well	10μL	10μL	2μL	10μL	10μL	12.5μL	5μL×4well
8-well chambered coverglass	10μL	10μL	2μL	10μL	10μL	12.5μL	10μL×2well

➢ Recommended cell density for each well plate size

Plate size	Cell density (upon inoculation onto the well plate*)	Amount of medium
6-well plate	0.4～2×10^5 cells/well	2.0 mL /well
24-well plate	1～5×10^4 cells/well	0.5 mL /well
96-well plate	0.25～1.25×10^4 cells/well	0.125 mL /well
8-well-chamberd coverglass	0.5～2.5×10^4 cells/well	0.2 mL /well

* Used for transfection under conditions of one-day culture and 40-60% confluency.

■ Methods for observation of the distribution (localization) of antibody introduced into viable cells

Cell fixation and staining with fluorescence-labeled secondary antibody (example of a protocol)

⇒ Refer to page 10

（2） Suspension cells

When delivery of floating cells is attempted, the mixture of HVJ-E and cells is centrifuged to increase the efficiency of introduction.

■ **Recommended antibody level**：0.1～0.5 mg/mL

⚠ If glycerol is included as a stabilizer in the antibody solution, the solution needs to be diluted to a glycerol concentration below 20 (v/v)%, in order to avoid reduction of efficiency of antibody incorporation into HVJ-E or cell function. Efficiency of antibody inclusion will not be affected by the presence of 1% BSA or 0.1% gelatin in the antibody solution.

■ **Cell density**（For 24-well plate／cell densities for plates of other sizes are given on page 9）:
1～5×10^5 cells/0.25mL of medium/tube (at the time of centrifugation).

■ **Protocol**

[1] Preparation (reconstitution) of ”HVJ-E suspension” and “Reagent Ⅰ solution”

Ice-cooled buffer (0.26 mL) is added to each tube containing “freeze-dried HVJ-E” or “freeze-dried Reagent Ⅰ”. The mixture is pipetted gently, with care taken to avoid bubbling. A homogeneous suspension is thus prepared and stored refrigerated. Immediately before use, HVJ-E suspension, other reagents, and tubes should be cooled adequately in an ice bath.
Storage conditions are given on page 5.

[2] Antibody incorporation into HVJ-E and introduction into cells （For the use of 24-well plates）

		Step	**Amount of reagent**
Incorporation	(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 10 μL
	(2)	Combination with “Reagent Ⅰ solution“ (Antibody Incorporation Enhancer) and agitation (tapping).	Reagent Ⅰ :10 μL
	(3)	Combination with “Reagent Ⅱ“ (Incorporation Reagent) and agitation[3] (tapping).	Reagent Ⅱ: 2 μL
	(4)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(5)	Combination with IgG antibody solution (0.1-0.5 mg/mL) and agitation (pipetted 20-30 times).	IgG solution: 10 μL (1-5 μg)
	(6)	Left to stand on ice for 5 minutes.	
	(7)*	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(8)*	Sediment suspended in the “Buffer” (pipetted 20-30 times).	Buffer: 10 μL
	(9)	Combination with “Reagent Ⅲ” (Introduction Enhancer) and agitation (tapping)	Reagent Ⅲ: 12.5 μL
	(10)	HVJ-E vector suspension is combined with the cells suspended in medium (1-5×10^5 cells /0.25 mL) in a tube. Serum in the medium does not affect the efficiency of antibody delivery into cells.	Suspension ((8) + (9)): 22.5 μL + Cell suspension: 0.25mL
	(11)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [4].	
	(12)	The supernatant is discarded. The cells are resuspended in 0.5 mL medium and transferred to a 24-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 0.5 mL (for one well)

- Steps (1) through (10) should be performed on ice.
- * Steps (7) and (8) may be skipped if the additive to the antibody solution affects neither cell viability nor cell function.

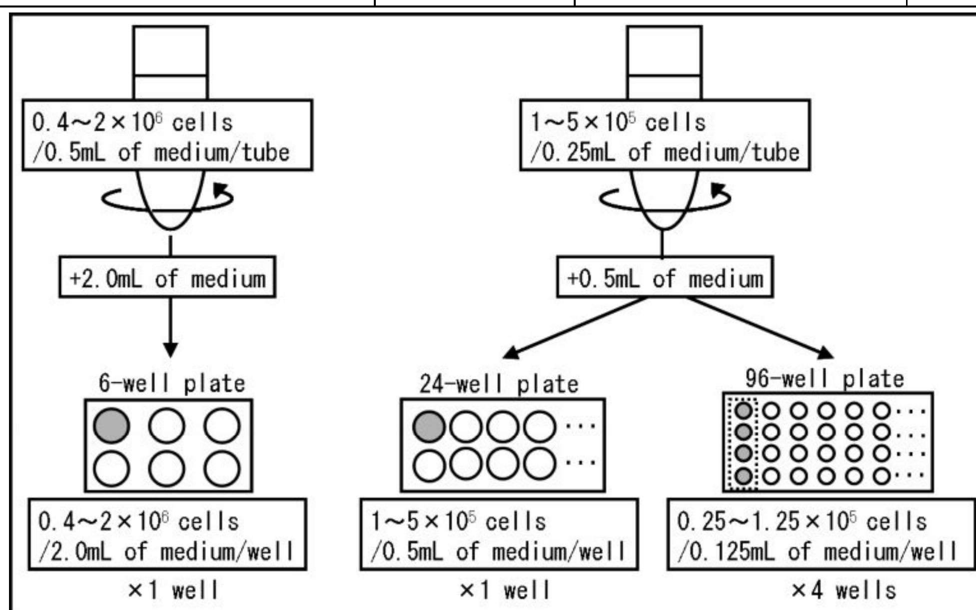
[3] The amount of “Reagent Ⅱ“ added should equal to 1/10 of the fluid volume before addition (Steps (1)+(2)).
[4] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

➢ Amount of reagent for each plate size

Plate size	Incorporation Step				Introduction Step			
	HVJ-E (1)	Reagent I (2)	Reagent II (3)	Antibody (5)	Buffer (8)	Reagent III (9)	Cell (10)	Medium for resuspension (12)
6-well	40μL	40μL	8μL	40μL	40μL	50μL	0.5mL	2.0 mL (2.0mL×1 well)
24-well	10μL	10μL	2μL	10μL	10μL	12.5μL	0.25mL	0.5 mL (0.5mL×1 well)
96-well	10μL	10μL	2μL	10μL	10μL	12.5μL	0.25mL	0.5 mL (0.125mL×4 well)
8-well chambered coverglass	10μL	10μL	2μL	10μL	10μL	12.5μL	0.25mL	0.5 mL (0.2mL×2 well)

➢ Recommended cell density for each well plate size

Plate size	Cell density・Amount of medium			
	Centrifugation (in a tube) (10)～(11)	Medium for resuspension (12)	Inoculation onto the well plate	
			Number of cells	Amount of medium
6-well plate	0.4～2.0×10^6 cells/0.5mL/tube	2.0mL	0.4～2.0×10^6 cells/well	2.0 mL/well
24-well plate	1～5×10^5 cells/0.25mL/tube	0.5mL	1.0～5.0×10^5 cells/well	0.5 mL/well
96-well plate			0.25～1.25×10^5 cells/well	0.125 mL/well
8-well-chamberd coverglass			0.5～2.5×10^5 cells/well	0.2 mL/well



HVJ–E vector preparation and introduction into cells

■ Methods for observation of the distribution (localization) of antibody introduced into viable cells

Cell fixation and staining with fluorescence-labeled secondary antibody (example of a protocol)

⇒ Refer to page 10

4. Manipulations for related experiments

Methods for observation of the distribution (localization) of antibody introduced into viable cells

Cell fixation and staining with fluorescence-labeled secondary antibody (example of a protocol)

Cells (40-60% confluent)

10%FCS/DMEM (8-wellchambered coverglass; Nunc LAB-TEK Cat No.155411)

↓Antibody delivery into living cells using *GenomONE-CAb* Antibody Delivery Reagent

↓incubation for 2 hours, 37℃, 5% CO_2

↓wash the cells, PBS(-)×2

↓fix the cells, 4% PFA; for 15min at room temperature

↓wash the cells, PBS(-)×2

↓0.2% Triton X-100, for 10 minutes at room temperature

↓wash the cells, PBS(-)×2

↓1% BSA/PBS, for 10 minutes at room temperature

↓AlexaFluor 488-Goat Anti-Mouse IgG, $F(ab')_2$ Fragment* (Invitrogen A11017), 500× dilution

↓wash the cells, PBS(-)×3

↓observation by confocal laser scanning microscopy

* Important Notice

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

5. Precautions and trouble shooting

(1) This kit is a reagent optimized for introduction of IgG antibodies. The efficiency of antibody incorporation into HVJ-E and the efficiency of antibody introduction into cells will be lower if this kit is used for IgM, IgA, IgE, IgY, single chain (scFv) or Fab antibodies compared to IgG antibodies.

(2) This kit is designed for introduction of non-labeled monoclonal antibodies into viable cells.
If this kit is used to introduce fluorescence-labeled antibody, polyclonal antibodies (purified IgG fractions) or antisera, nonspecific binding to cell surfaces may occur, possibly reducing the efficiency of antibody introduction into cells. This can be alleviated with the following countermeasures

- Use of Reagent III at a concentration equal to 1/10 of the prescribed level
- Reducing the concentration of antibody to be incorporated into HVJ-E to 1/2 to 1/10 of the original level
- Wash the cells with 0.01% Trypsin/PBS(-) for 5 minutes. at room temperature prior to fluorescence observation

(3) Precautions regarding observation of the distribution of introduced antibody using fluorescence-labeled secondary antibody after fixation of cells

(3)-1 $F(ab')_2$ fragment must be used as the labeled secondary antibody for fluorescent staining. If whole antibody is used, unspecific binding may occur, making it impossible for specific chromatic responses to appear.

(3)-2 Small debris emitting fluorescence may be noted on the bottom of the culture dish or plates. In this case, the debris can be reduced in the following ways.

- Use of Reagent III at a concentration equal to 1/10 of the prescribed level
- Renewal of medium 30 minutes after treatment of the cells with antibody-incorporated HVE-J vector (if this is unsuccessful, the medium is renewed again 2 hours later)
- Wash the cells with 0.01% Trypsin/PBS(-) for 5 minutes at room temperature just before fixation of the cells
- After introduction of the antibody into adhesion cells, the cells are freed by trypsin treatment and inoculated into another culture dishes or plates
- After introduction of the antibody into suspension cells, HVJ-E vector is washed out by centrifugation, thereafter inoculated into culture dishes or plates

(4) Causes of and countermeasures when dealing with low efficiency of antibody introduction or inadequate suppression of function

(4)-1 Additives contained in the antibody solution may reduce the efficiency of antibody incorporation into HVJ-E.

- If glycerol is contained in the antibody solution to be used for antibody incorporation, its concentration should not exceed 20%
- The presence of 1%BSA, 0.1% gelatin or 0.1% NaN_3 (sodium azide) does not affect the efficiency of antibody incorporation

(4)-2 Additives in the antibody solution (NaN_3, thimerosal, etc.) may affect cell proliferative activity or other functions. Each test system needs to be checked for additives in advance.

(4)-3 If no effects of additives are expected, try antibody incorporation into HVJ-E with an antibody concentration 2-5 times as high as normal.

(4)-4 If the reactivity of the antibody against intracellular naïve antigen is low, suppression of function may not be observed even after antibody introduction. In such cases, check the antibody reactivity (binding or neutralization activity) by immunoprecipitation, etc., or try another antibody, as needed.

(5) Causes of and countermeasures when cell toxicity is marked
Try reducing the level (amount) of HVJ-E vector to be treated with cells to 1/2 to 1/4 of the original level. It is possible that the introduced antibody itself affects cell proliferation.

6. Types of antibodies to which this kit is applicable

We have verified that with this kit the following types of IgG may be introduced into the cytoplasm of Hs68 cells (human foreskin fibroblasts: ATCC CRL-1635).

Species	IgG subtype *1	Incorporation efficiency of antibody into HVJ-E *2	Introduction efficiency of antibody into Hs68 cells *3
Mouse	pAb	◎	△
	IgG1	◎	◎
	IgG2a	○	○
	IgG2b	◎	△
	IgG3	○	△
Rat	pAb	△	△
	IgG1	◎	○
	IgG2a	△	△
	IgG2b	○	○
	IgG2c	△	△
Human	IgG1	◎	○
	IgG2	◎	○
	IgG3	△	△
	IgG4	◎	○
Rabbit	pAb	○	○
Goat	pAb	△	△

*1: pAb: polyclonal antibody, others: monoclonal antibody (control IgG)
*2: Incorporation efficiency of antibody into HVJ-E:
◎; more than 40%, ○; 20-40%, △; less than 20%
*3: Introduction efficiency of antibody into Hs68 cells:
◎; Easy to introduce, ○; Possible to introduce, △; Not efficient but possible to introduce

(Caution)
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 countermeasures indicated on page 11.

7. Advantages of intracellular antibody introduction and possible applications

(1) Advantages of intracellular antibody introduction (differences from existing knockout method)

- ✓ Unlike post-transcription 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.

(2) Possible applications

- **Analysis of intracellular function**

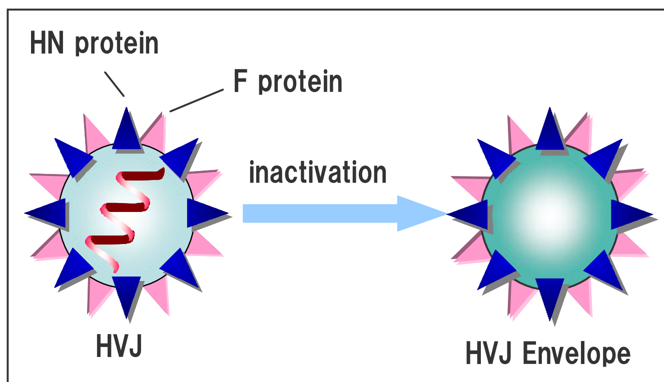
  An antibody is introduced into viable cells ---
  - ✓ to examine the distribution of the target molecule
  - ✓ to suppress and clarify the function of target molecules

- **Screening of antibodies reacting to intracellular antigens**

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

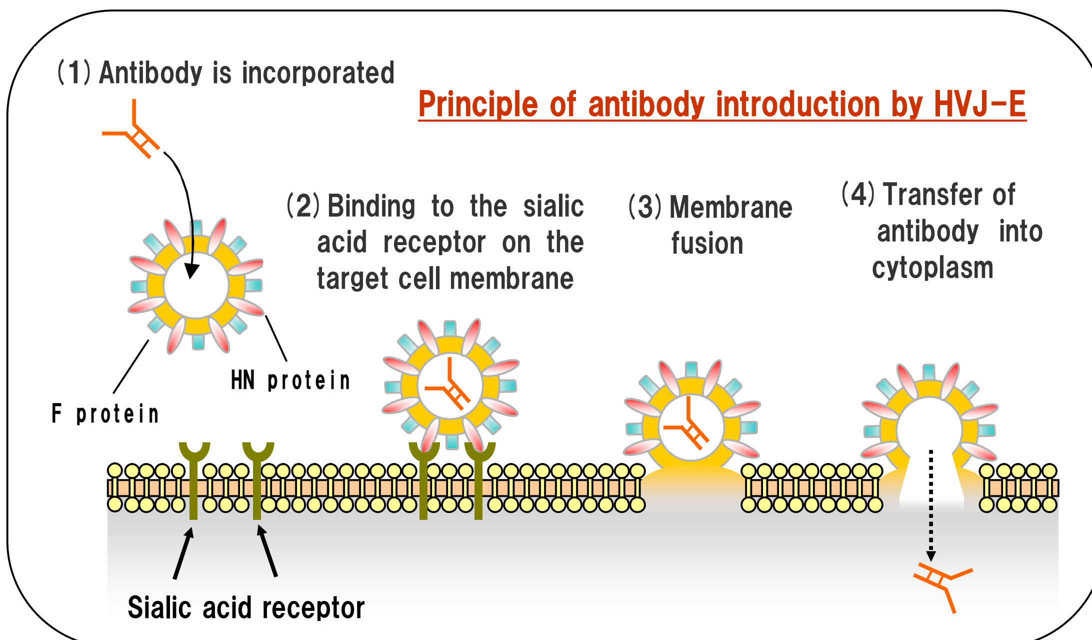
- **Other possible applications**
  - ✓ Proteins with tag are delivered into living cells (delivery of tagged-proteins in combination with anti-tag antibodies)
  - ✓ Live cell imaging is performed
  - ✓ New agents for testing and diagnosis are developed using antibodies capable of detecting target molecules in living cells
  - ✓ Next-generation antibody-based drugs are created, which exert therapeutic effects through acting on intracellular target molecules

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)



NOTES

ISHIHARA SANGYO KAISHA, LTD.

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

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