HVJ Envelope VECTOR KIT

GenomONE™ - Neo

Instruction Manual (Ver. 1.1)

- **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™-Neo for transfection with genes, proteins, etc. The methods described here yield reasonable efficiency of transfection, though optimal conditions of transfection can vary depending on cell type. It is advisable to optimize the conditions of transfection, 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. HVJ-E 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. HVJ-E should be stored at -80°C. After thawing, HVJ-E requires refrigerated storage 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. Outline

1-1: Principle of transfection

GenomONE™-Neo is a non-viral reagent for transfection developed on the basis of the technology described in the following paper.

- Kaneda, Y., *et al.*: Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. *Molecular Therapy*, 6, 219-226 (2002).
- Kaneda, Y., *et al.*: New vector innovation for drug delivery: development of fusigenic non-viral particles. *Curr. Drug Targets*, 4, 599-602 (2003).

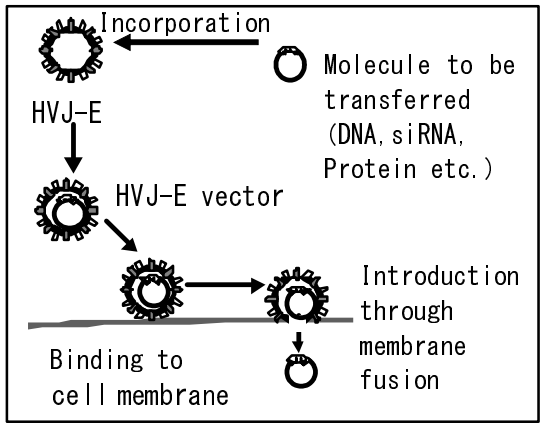

Fig.1 Principle of transfection

The molecule to be transferred (DNA, protein, antisense oligonucleotide, siRNA, etc.) is incorporated in the HVJ-Envelope (HVJ-E) to yield an HVJ-E vector, which is then introduced into the target cell or tissue, making use of the membrane-fusion activity of fusion (F) protein (Fig. 1).

*HVJ: Hemagglutinating Virus of Japan, synonym for Sendai Virus (SeV).

1-2: Specifications

Cat. #	**HVJ-E(N)** (inactivated HVJ) 0.25 mL /vial	**Reagent A** (enhancer for incorporation) 0.5 mL/vial	**Reagent B** (reagent for incorporation) 0.3 mL/vial	**Reagent C** (enhancer for introduction) 1.0 mL/vial	**Buffer** (for dilution) 6.5 mL/vial
GN001	1 vial	1 vial	1 vial	1 vial	1 vial
GN004	4 vials	1 vial	1 vial	1 vial	1 vial
GN016	16 vials	4 vials	4 vials	4 vials	4 vials
GN040	40 vials	10 vials	10 vials	10 vials	10 vials

Storage:

Reagent A, B, C and Buffer: Refrigerated at 2-8°C.

HVJ-E: Store at -80 °C. For details, Refer to the following “Thawing method of HVJ-E” and “Storage, stability, and quality assurance”.

[Role of each reagent]

- 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 A: A positively-charged peptide which increases the affinity between the target molecule and HVJ-E and thus facilitates incorporation of the molecule into HVJ-E.
- Reagent B: Increases permeability across the HVJ-E membrane.
- Reagent C: A positively-charged peptide which increases affinity between the molecule-bearing HVJ-E (HVJ-E vector) and the cell (or tissue) and thus increases the efficiency of transfection.
- Buffer: Neutral buffer of physiological concentration used for diluting HVJ-E or other purposes.

[Thawing method of HVJ-E]

- Quickly thaw frozen HVJ-E into a semi-thawed state in a thermostatic water bath at 34 to 37°C and immediately transfer it on ice. Do not completely thaw at 34 to 37°C.

[Storage, stability, and quality assurance]

Store at -80 °C. After thawing, HVJ-E requires refrigerated storage at 2-8°C and should be used within 2 weeks. For extended storage, refreeze in working aliquots at -80°C for up to 3 months. Thawing after refreezing 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 and safety]

- 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:

Kaneda, Y. *et al.*: "Non-Viral Vectors for Gene Therapy", Advances in Genetics, Vol. 53, pp 308-332, Ed. Huang Leaf, Hung Mien-Chie, Wagner Ernst, Academic Press (2005).

Related article:

Prior, C. et al.: BioPharm, 22-33 (Oct. 1996)

! 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.

- Endotoxin level has been confirmed to be less than 2.5 EU/mL (Limulus amebocyte lysate gel clot assay).
- Expression of the gene introduced in cultured cells (BHK-21; ATCC CCL-10) in the presence of serum has been confirmed.

[Frequency of use]

- If used with the method described in this package insert, the product can be used for transfection as follows (with a 6-well plate).

Cat. #	No. of HVJ-E vials per kit	Cargo to be transfected	
		Plasmid DNA, ODN, protein	siRNA (oligo-type)
GN001	1	6 assays (wells)	25-50 assays (wells)
GN004	4	25 assays (wells)	100-200 assays (wells)
GN016	16	100 assays (wells)	400-800 assays (wells)
GN040	40	250 assays (wells)	1000-2000 assays (wells)

2. Methods described in this package insert

This package insert describes standard methods for use of this product for transfection of adherent cells, suspension cells, and animals. It also describes protocols for rapid processing of many samples.

Diverse methods are available for use depending on the molecule to be transferred and the destination of transfer. This package insert describes individual steps of transfection (inclusion and transfer) for each molecule to be transferred into each destination (adhesive cells, floating cells, and animals). The inclusion step differs depending on the concentration and type of molecule to be transferred.

2-1: Definition of quantity (AU: Assay Unit)

Amounts of HVJ-E are expressed in AU (Assay Units). One AU is defined as the standard amount (40 μL) used for transfection with plasmid DNA, using a 6-well plate.

2-2: Recommended cell density for each well plate size

The conditions shown in the protocol pertain to cases in which 6-well plates is used. The cell densities for well plates other than 6-well plates are given below.

2-2-1 : Adherent cells

Plate	Cell density (upon inoculation onto the well plate*)
6-well plate	0.4 - 2.0 × 10^5 cells/2.0 mL of medium/well
24-well plate	1.0～5.0 × 10^4 cells/0.5mL of medium/well
96-well plate	0.25～1.25 × 10^4 cells/0.125mL of medium/well

*Used for transfection under conditions of one-day culture and 50-80% confluency.

2-2-2 : Suspension cells

When transfection of suspension cells is performed, the cells are combined with HVJ-E vector in a tube, and the mixture is centrifuged to induce contact between the cells and the vector, leading to transfection.

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

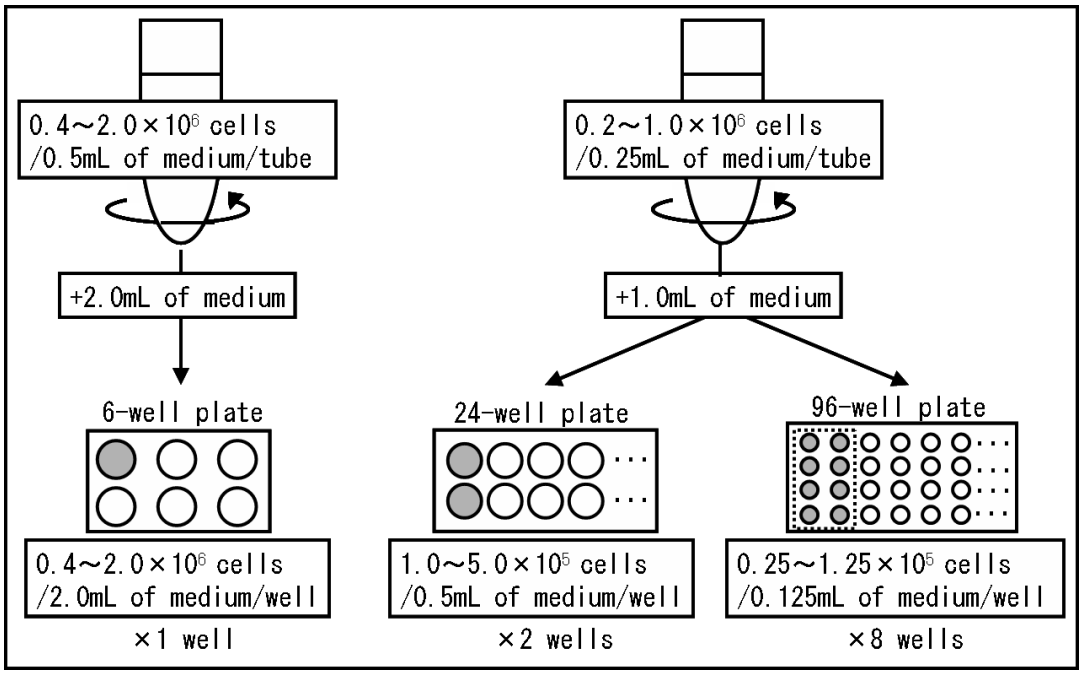

Fig. 2 : Transfection of suspension cells

3. Transfection of adherent cells

3-1: Transfer of plasmid DNA

3-1-1: Recommended protocol

The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended DNA/TE solution concentration: 2-4 μg/μL
- Cell density: 0.4-2.0 × 10^5 cells/2.0 mL of medium/well of 6-well plate
- Protocol (Method 2)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(3)	Suspension in DNA/TE solution (pipetted 20-30 times or more until the suspension becomes uniformly white)	DNA/TE solution: 10-20 μL
	(4)	Combination with Reagent B and agitation[1] (tapping)	Reagent B: 1-2 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[2]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[3] (tapping)	Reagent C: 5 μL
	(8)	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)[4]	Suspension ((6) + (7)): 35 μL
	(9)	Incubated at 37°C under 5%CO_2.	

Steps (1) through (7) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step			Introduction Step		
	HVJ-E (1)	DNA/TE soln. (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Amount of HVJ-E vector to be treated (8)
6-well	1AU (40μL)	20μL	2μL	30μL	5μL	35μL×1 well
24-well	0.5AU (20μL)	10μL	1μL	15μL	2.5μL	8μL×2 wells
96-well	0.5AU (20μL)	10μL	1μL	15μL	2.5μL	2μL×8 wells

[1] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[2] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.
[3] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.
[4] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (6) + (7)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

3-1-2: Protocol for use of low concentrations of DNA

If the concentration of DNA/TE solution is lower than the recommended concentration (0.5-2 μg/μL), Reagent A is used to promote inclusion of DNA into HVJ-E. The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended DNA/TE concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^5 cells/2.0 mL of medium/well of 6-well plate
- Protocol (Method 1)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent A and agitation (tapping). Left to stand on ice for 5 minutes.	Reagent A: 10 μL
	(3)	Suspension in DNA/TE solution (tapping)	DNA/TE solution: 10 μL
	(4)	Combination with Reagent B and agitation[5] (tapping)	Reagent B: 6 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[6]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[7] (tapping)	Reagent C: 5 μL
	(8)	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)[8]	Suspension ((6) + (7)): 35 μL
	(9)	Incubated at 37°C under 5%CO_2.	

Steps (1) through (7) should be performed on ice.

- Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step		
	HVJ-E (1)	Reagent A (2)	DNA/TE soln. (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Amount of HVJ-E vector to be treated (8)
6-well	1AU (40μL)	10μL	10μL	6μL	30μL	5μL	35μL×1 well
24-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	8μL×2 wells
96-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	2μL×8 wells

[5] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[6] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.
[7] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.
[8] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (6) + (7)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

3-2: Transfer of siRNA

siRNA (oligo-type) can be used at low concentrations since it exerts activity in cytoplasm and its activity is highly specific. Furthermore, the amount of HVJ-E used can be reduced to 1/4-1/8 (0.25-0.125 AU) of the amount needed with plasmid DNA. The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended siRNA (oligo-type) concentration: 0.1-0.5 μg/μL
- Cell density: 0.4-2.0 × 10^5 cells/2.0 mL of medium/well of 6-well plate
- Protocol (Method for siRNA)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 0.25AU (10 μL)
	(2)	Combination with siRNA solution and agitation[9] (tapping)	siRNA solution: 10 μL
	(3)	Combination with Reagent B and agitation[10] (tapping)	Reagent B: 2 μL
	(4)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(5)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[11]	Buffer: 30 μL
	(6)	Combination with Reagent C and agitation[12] (tapping)	Reagent C: 5 μL
	(7)	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)[13]	Suspension ((5) + (6)): 35 μL
	(8)	Incubated at 37°C under 5%CO_2.	

Steps (1) through (6) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step			Introduction Step		
	HVJ-E (1)	siRNA soln. (2)	Reagent B (3)	Buffer (5)	Reagent C (6)	Amount of HVJ-E vector to be treated (7)
6-well	0.25AU (10μL)	10μL	2μL	30μL	5μL	35μL×1 well
24-well	0.125AU (5μL)	5μL	1μL	15μL	2.5μL	8μL×2 wells
96-well	0.125AU (5μL)	5μL	1μL	15μL	2.5μL	2μL×8 wells

● One-point advice

The optimal amount of HVJ-E can vary depending on the type of cell used or the target gene. If efficiency of transfer or knock-down is low, adjust the amount of HVJ-E used in Step 1 within the range between 1 AU (40 μL) and 0.125 AU (5 μL) to optimize conditions. At that time, the amount of Reagent B added needs to be adjusted to 1/10 of the volume of the fluid before addition (Steps 1 + 2).

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[9] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[10] The amount of Reagent B added is equal to 1/10 of the fluid volume before addition (Step 3).
[11] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.
[12] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 5) should be adjusted to make the final volume of the suspension equal to 35 μL.
[13] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (6) + (7)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

3-3: Transfer of antisense oligo/decoy-oligo (ODN)

The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended ODN concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^5 cells/2.0 mL of medium/well of 6-well plate
- Protocol (Method 1)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent A and agitation (tapping). Left to stand on ice for 5 minutes.	Reagent A: 10 μL
	(3)	Suspension in DNA/TE solution (tapping)	DNA/TE solution: 10 μL
	(4)	Combination with Reagent B and agitation[14] (tapping)	Reagent B: 6 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[15]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[16] (tapping)	Reagent C: 5 μL
	(8)	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)[17]	Suspension ((6) + (7)): 35 μL
	(9)	Incubated at 37°C under 5%CO_2.	

Steps (1) through (7) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step		
	HVJ-E (1)	Reagent A (2)	ODN soln. (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Amount of HVJ-E vector to be treated (8)
6-well	1AU (40μL)	10μL	10μL	6μL	30μL	5μL	35μL×1 well
24-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	8μL×2 wells
96-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	2μL×8 wells

[14] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[15] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.
[16] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.
[17] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (6) + (7)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

3-4: Transfer of protein

The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended protein concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^5 cells/2.0 mL of medium/well of 6-well plate
- Protocol (Method 1)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(3)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[18]	Buffer: 40 μL
	(4)	Combination with Reagent A and agitation (tapping). Left to stand on ice for 5 minutes.	Reagent A: 10 μL
	(5)	Combination with protein solution and agitation (tapping).	Protein solution: 10 μL
	(6)	Combination with Reagent B and agitation[19] (tapping)	Reagent B: 6 μL
	(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 or more until the suspension becomes uniformly white)	Buffer: 30 μL
	(9)	Combination with Reagent C and agitation[20] (tapping)	Reagent C: 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)[21]	Suspension ((8) + (9)): 35 μL
	(11)	Incubated at 37°C under 5%CO_2.	

Steps (1) through (9) should be performed on ice.

Steps (2) and (3) are designed to eliminate the preservatives contained in the freeze-dried HVJ-E.

■ Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step		
	HVJ-E (1)	Reagent A (4)	Protein soln. (5)	Reagent B (6)	Buffer (8)	Reagent C (9)	Amount of HVJ-E vector to be treated (10)
6-well	1AU (40μL)	10μL	10μL	6μL	30μL	5μL	35μL×1 well
24-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	8μL×2 wells
96-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	2μL×8 wells

● One-point advice

When positively-charged protein is used, try either reducing the amount of Reagent A added in Incorporation Step (2) (to 1/2 to 1/8) or skipping the addition of Reagent A. At that time, the amount of Reagent B added in Step (4) needs to be adjusted to 1/10 of the volume before addition (Steps (3) + (4) + (5)).

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[18] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[19] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).

[20] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 8) should be adjusted to make the final volume of the suspension equal to 35 μL.

[21] 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 3 hours of exposure.

3-5: Troubleshooting

■ Low efficiency of transfection

Efficiency may be increased by the following measures:

- Increase the amount of Reagent C two- to four-fold compared to the standard amount.
- Reduce the amount of medium used for transfection and increase the concentrations of HVJ-E vector and Reagent C.
- Centrifuge the mixture of HVJ-E vector and cells in the plate at 1,500-3,000 rpm for 10-60 minutes at a temperature of 35°C (4°C to room temperature for some types of cells).
- Check the purity of nucleic acid. Plasmid DNA used for transfection should be of high quality. Endotoxin level should also be reduced by using appropriate purification tools.

■ High cytotoxicity

If any sign of cytotoxicity is noted, cytotoxicity may be reduced by the following measures:

- Wash the HVJ-E vector 10 minutes to 3 hours after addition to the cells, and renew the medium.
- Reduce the amount of HVJ-E used or the amount of HVJ-E vector added to the medium.
- Endotoxin level should also be reduced by using appropriate removing tools.

4. Transfection of suspension cells

4-1: Transfer of plasmid DNA

4-1-1: Recommended protocol

When transfection of floating cells is attempted, the mixture of HVJ-E and cells is centrifuged to increase the efficiency of exposure. The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended DNA/TE solution concentration: 2-4 μg/μL
- Cell density: 0.4-2.0 × 10^6 cells/0.5 mL of medium/tube
- Protocol (Method 2, modified)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 ºC)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(3)	Suspension in DNA/TE solution (pipetted 20-30 times or more until the suspension becomes uniformly white)	DNA/TE solution: 10-20 μL
	(4)	Combination with Reagent B and agitation[22] (tapping)	Reagent B: 1-2 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[23]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[24] (tapping)	Reagent C: 5 μL
	(8)	HVJ-E vector suspension is combined with the cells suspended in medium (0.5 mL) in a tube.	Suspension (Steps (6) + (7)):35 μL + Cell: 0.5 mL
	(9)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [25].	
	(10)	The supernatant is discarded. The cells are resuspended in 2.0 mL medium and transferred to a 6-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 2.0 mL

Steps (1) through (7) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step			Introduction Step			
	HVJ-E (1)	DNA/TE (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Cell (8)	Medium for resuspension (10)
6-well	1AU (40μL)	20μL	2μL	30μL	5μL	0.5mL	2.0mL (2.0mL×1well)
24-well	0.5AU (20μL)	10μL	1μL	15μL	2.5μL	0.25mL	1.0mL (0.5mL×2 wells)
96-well	0.5AU (20μL)	10μL	1μL	15μL	2.5μL	0.25mL	1.0mL (0.125mL×8 wells)

[22] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).

[23] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[24] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.

[25] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

4-1-2 : Protocol for use of low concentrations of DNA

If the concentration of DNA/TE solution is lower than the recommended concentration (0.5-2 μg/μL), Reagent A is used to promote inclusion of DNA into HVJ-E. The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended DNA/TE concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^6 cells/0.5 mL of medium/tube
- Protocol (Method 1)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent A and agitation (tapping). Store on ice for 5 minutes.	Reagent A: 10 μL
	(3)	Suspension in DNA/TE solution (tapping)	DNA/TE solution: 10 μL
	(4)	Combination with Reagent B and agitation[26] (tapping)	Reagent B: 6 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[27]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[28] (tapping)	Reagent C: 5 μL
	(8)	HVJ-E vector suspension is combined with the cells suspended in medium (0.5 mL) in a tube.	Suspension (Steps (6) + (7)):35 μL + Cell: 0.5 mL
	(9)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [29].	
	(10)	The supernatant is discarded. The cells are resuspended in 2.0 mL medium and transferred to a 6-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 2.0 mL

Steps (1) through (7) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step			
	HVJ-E (1)	Reagent A (2)	DNA/TE Soln. (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Cell (8)	Medium for resuspension (10)
6-well	1AU (40μL)	10μL	10μL	6μL	30μL	5μL	0.5mL	2.0mL (2.0mL×1 well)
24-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.5mL×2 wells)
96-well	0.5AU (20μL)	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.125mL×8 wells)

[26] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).

[27] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[28] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.

[29] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

4-2: Transfer of siRNA

siRNA (oligo-type) can be used at low concentrations since it exerts activity in cytoplasm and its activity is highly specific. Furthermore, the amount of HVJ-E used can be reduced to 1/4-1/8 (0.25-0.125 AU) of the amount needed with plasmid DNA. The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended siRNA (oligo-type) concentration: 0.1-0.5 μg/μL
- Cell density: 0.4-2.0 × 10^6 cells/0.5 mL of medium/tube
- Protocol (Method for siRNA, modified)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 0.25AU (10 μL)
	(2)	Combination with siRNA solution and agitation (tapping)	siRNA solution: 10 μL
	(3)	Combination with Reagent B and agitation[30] (tapping)	Reagent B: 2 μL
	(4)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(5)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[31]	Buffer: 30 μL
	(6)	Combination with Reagent C and agitation[32] (tapping)	Reagent C: 5 μL
	(7)	HVJ-E vector suspension is combined with the cells suspended in medium (0.5 mL) in a tube.	Suspension (Steps (5) + (6)):35 μL + Cell: 0.5 mL
	(8)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [33].	
	(9)	The supernatant is discarded. The cells are resuspended in 2.0 mL medium and transferred to a 6-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 2.0 mL

Steps (1) through (6) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step			Introduction Step			
	HVJ-E (1)	siRNA soln. (2)	Reagent B (3)	Buffer (5)	Reagent C (6)	Cell (7)	Medium for resuspension (9)
6-well	0.25AU(10μL)	10μL	2μL	30μL	5μL	0.5mL	2.0mL (2.0mL×1 well)
24-well	0.125AU(5μL)	5μL	1μL	15μL	2.5μL	0.25mL	1.0mL (0.5mL×2 wells)
96-well	0.125AU(5μL)	5μL	1μL	15μL	2.5μL	0.25mL	1.0mL (0.125mL×8 wells)

● One-point advice

The optimal amount of HVJ-E can vary depending on the type of cell used or the target gene. If efficiency of transfer or knock-down is low, adjust the amount of HVJ-E used in Step 1 within the range between 1 AU (40 μL) and 0.125 AU (5 μL) to optimize conditions. At that time, the amount of Reagent B added needs to be adjusted to 1/10 of the volume of the fluid before addition (Steps (1) + (2)).

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[30] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).

[31] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[32] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 5) should be adjusted to make the final volume of the suspension equal to 35 μL.

[33] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

4-3: Transfer of antisense oligo/decoy-oligo (ODN)

The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended ODN concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^6 cells/0.5 mL of medium/tube
- Protocol (Method 1, modified)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent A and agitation (tapping). Left to stand on ice for 5 minutes.	Reagent A: 10 μL
	(3)	Suspension in ODN solution (tapping)	DNA/TE solution: 10 μL
	(4)	Combination with Reagent B and agitation[34] (tapping)	Reagent B: 6 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
Introduction	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[35]	Buffer: 30 μL
	(7)	Combination with Reagent C and agitation[36] (tapping)	Reagent C: 5 μL
	(8)	HVJ-E vector suspension is combined with the cells suspended in medium (0.5 mL) in a tube.	Suspension (Steps (6) + (7)):35 μL + Cell: 0.5 mL
	(9)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [37].	
	(10)	The supernatant is discarded. The cells are resuspended in 2.0 mL medium and transferred to a 6-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 2.0 mL

Steps (1) through (7) should be performed on ice.

■ Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step			
	HVJ-E (1)	Reagent A (2)	ODN soln. (3)	Reagent B (4)	Buffer (6)	Reagent C (7)	Cell (8)	Medium for resuspension (10)
6-well	1AU（40μL）	10μL	10μL	6μL	30μL	5μL	0.5mL	2.0mL (2.0mL×1well)
24-well	0.5AU（20μL）	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.5mL×2 wells)
96-well	0.5AU（20μL）	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.125mL×8 wells)

[34] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[35] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.
[36] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 6) should be adjusted to make the final volume of the suspension equal to 35 μL.
[37] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

4-4: Transfer of protein

The protocol shown below pertains to the use of 6-well plates. The cell densities for plates of other sizes are given on Page 5.

- Recommended protein concentration: 0.5-2 μg/μL
- Cell density: 0.4-2.0 × 10^6 cells/0.5 mL of medium/tube
- Protocol (Method 1, modified)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Centrifugation at 10,000 g (10,000-12,000 rpm) for 5 minutes at 4°C. Supernatant is discarded.	
	(3)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[38]	Buffer: 40 μL
	(4)	Combination with Reagent A and agitation (tapping). Left to stand on ice for 5 minutes.	Reagent A: 10 μL
	(5)	Combination with protein solution and agitation (tapping).	Protein solution: 10 μL
	(6)	Combination with Reagent B and agitation[39] (tapping)	Reagent B: 6 μL
	(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 or more until the suspension becomes uniformly white)	Buffer: 30 μL
	(9)	Combination with Reagent C and agitation[40] (tapping)	Reagent C: 5 μL
	(10)	HVJ-E vector suspension is combined with the cells suspended in medium (0.5 mL) in a tube.	Suspension (Steps (8) + (9)):35 μL + Cell: 0.5 mL
	(11)	Centrifugation at 2,000-12,000 rpm for 10-30 minutes at 4-35°C [41].	
	(12)	The supernatant is discarded. The cells are resuspended in 2.0 mL medium and transferred to a 6-well plate for incubation at 37°C under 5%CO_2.	Medium for resuspension: 2.0 mL

Steps (1) through (9) should be performed on ice.
Steps (2) and (3) are designed to eliminate the preservatives contained in the freeze-dried HVJ-E.

■ Amount of reagent for each plate

Plate size	Incorporation Step				Introduction Step			
	HVJ-E (1)	Reagent A (4)	Protein soln. (5)	Reagent B (6)	Buffer (8)	Reagent C (9)	Cell (10)	Medium for resuspension (12)
6-well	1AU(40μL)	10μL	10μL	6μL	30μL	5μL	0.5mL	2.0mL (2.0mL×1well)
24-well	0.5AU(20μL)	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.5mL×2 wells)
96-well	0.5AU(20μL)	5μL	5μL	3μL	15μL	2.5μL	0.25mL	1.0mL (0.125mL×8 wells)

● One-point advice

When positively-charged protein is used, try either reducing the amount of Reagent A added in Incorporation Step (2) (to 1/2 to 1/8) or skipping the addition of Reagent A. At that time, the amount of Reagent B added in Step (4) needs to be adjusted to 1/10 of the volume before addition (Steps (3) + (4) + (5)).

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[38] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[39] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).

[40] The optimal concentration of Reagent C can vary depending on the type of cell. It is advisable to appropriately adjust the amount of Reagent C added (range: 2.5-25 μL) depending on the type of cell used. The volume of buffer (Step 8) should be adjusted to make the final volume of the suspension equal to 35 μL.

[41] The conditions of centrifugation (rate of rotation, temperature, and duration) may be adjusted within the range not causing cell damage.

4-5: Troubleshooting

■ Low efficiency of transfection

Efficiency may be increased by the following measures:

- Increase the amount of Reagent C to two- to four-fold to the standard amount.
- Reduce the amount of medium used for transfection and increase the concentrations of HVJ-E vector and Reagent C.
- Extend the duration of centrifugation of the mixture of HVJ-E vector and cells to about 60 minutes.
- Check the purity of nucleic acid. Plasmid DNA used for transfection should be of high quality. Endotoxin level should also be reduced by using appropriate purification tools.

■ High cytotoxicity

If any sign of cytotoxicity is noted, cytotoxicity may be reduced by the following measures:

- Shorten the duration of centrifugation after the addition of HVJ-E vector to the cells to the minimum (10 minutes) and set the temperature during centrifugation at 4°C.
- Reduce the amount of HVJ-E used or the amount of HVJ-E vector added to the medium.
- Reduce the amount of Reagent C added (or skip use of this reagent).
- Endotoxin level should also be reduced by using appropriate removing tools.

5. Transfection of laboratory animals (*in vivo*)

The protocol shown below pertains to an example in which transfection of mouse organs or tissue (direct injection to organs or tissue) in vivo is attempted. The method of administration, dose level, etc., can vary markedly depending on the species of animals, the type or location of the target organ, and other factors. These conditions may need to be adjusted in individual cases.

5-1: Transfer of plasmid DNA or siRNA

- Recommended DNA/TE concentration: 1 μg/μL
- Recommended siRNA concentration: 1 μg/μL
- Protocol (In vivo Method M)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 °C)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent B and agitation[42] (tapping)	Reagent B: 4 μL
	(3)	Centrifugation at 10,000 g (10,000-12,000 rpm) and 4°C for 10 minutes. Supernatant is discarded.	
	(4)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[43]	Buffer, physiological saline, or the like: 10 μL
	(5)	Combination with DNA/TE solution or siRNA solution and agitation (tapping)	DNA/TE or siRNA solution: 10 μL
	(6)	Left to stand for 5 minutes	
Administration	(7)	HVJ-E vector suspension administered to animals (after dilution with physiological saline, etc., as needed)	Dose level: Adjusted tailored to the objective
	(8)	Subsequent evaluation (preparation of specimens, microscopy, etc.)	

Steps (1) through (9) should be performed on ice.

● One-point advice

- The amount of HVJ-E is approximately 1-2 AU for mice and 5-10 AU for rats. The amount of each reagent used in subsequent steps also needs to be adjusted in proportion to the amount of HVJ-E used.
- Depending on the features of the experimental system (the site of administration, route of administration, etc.), dose level must be adjusted appropriately by adding buffer, physiological saline, or the like to the HVJ-E vector suspension (Step (6)).
- When this product is used in animals, it is as a rule advisable to skip treatment with Reagent C. If the substance introduced needs to be retained in tissue near the site of injection, you may add Reagent C to HVJ-E vector suspension (Step (6)).
- Because HVJ-E can be easily adsorbed onto blood cells and be inactivated in vivo, it is advisable to select a route of administration involving less exposure to blood (direct injection to organs or tissue is recommended) or to perform perfusion of the animal prior to administration.

[42] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[43] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

5-2: Transfer of antisense oligo/decoy-oligo (ODN) and protein

- Recommended ODN concentration: 0.5-2 μg/μL
- Recommended protein concentration: 0.5-2 μg/μL
- Protocol (In vivo Method 1)

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (keep 0-8 ºC)	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1AU (40 μL)
	(2)	Combination with Reagent A and agitation (tapping). Left to stand for 5 minutes.	Reagent A: 10 μL
	(3)	Combination with ODN or protein solution and agitation (tapping)	ODN or protein solution: 10 μL
	(4)	Combination with Reagent B and agitation[44] (tapping)	Reagent B: 6 μL
	(5)	Centrifugation at 10,000 g (10,000-12,000 rpm) and 4°C for 5 minutes. Supernatant is discarded.	
Administration	(6)	Sediment suspended in the buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[45]	Use of a buffer, physiological saline, or the like, tailored to the experimental system.
	(7)	HVJ-E vector suspension administered to animals (after dilution with physiological saline, etc., as needed)	Dose level: Adjusted depending on the objective
	(8)	Subsequent evaluation (preparation of specimens, microscopy, etc.)	

Steps (1) through (6) should be performed on ice.

For incorporation of protein, the preservatives must to be removed by centrifugation in accordance with Steps 1 through 3 of incorporation procedure (Page 10).

● One-point advice

- The amount of HVJ-E is approximately 1-2 AU for mice and 5-10 AU for rats. The amount of each reagent used in subsequent steps also needs to be adjusted in proportion to the amount of HVJ-E used.
- Depending on the features of the experimental system (the site of administration, route of administration, etc.), dose level must be adjusted appropriately by adding buffer, physiological saline, or the like to the HVJ-E vector suspension (Step (6)).
- When this product is used in animals, it is as a rule advisable to skip treatment with Reagent C. If the substance introduced needs to be retained in tissue near the site of injection, you may add Reagent C to HVJ-E vector suspension (Step (6)).
- Because HVJ-E can be easily adsorbed onto blood cells and be inactivated in vivo, it is advisable to select a route of administration involving less exposure to blood (direct injection to organs or tissue is recommended) or to perform perfusion of the animal prior to administration.
- In case of abnormal turbidity or non-homogeneous sedimentation in the solution prepared in Step (3), try either increasing the purity of the ODN/protein to be added or reducing the amount of Reagent A (Step (2)) to 1/2-1/8 or skipping use of Reagent A. At that time, the amount of Reagent B (Step (4)) needs to be adjusted to 1/10 of the volume before addition (Steps (1) + (2) + (3)).
- When positively-charged protein is used, try either reducing the amount of Reagent A added in Inclusion Step (2) (to 1/2 to 1/8) or skipping the addition of Reagent A. At that time, the amount of Reagent B added in Step (4) needs to be adjusted to 1/10 of the volume before addition (Steps (1) + (2) + (3)).

[44] The amount of Reagent B added should equal to 1/10 of the fluid volume before addition (Step 3).
[45] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

6. Rapid transfection for multiple types of and numerous samples

Treatment of HVJ-E with Reagent B prior to inclusion of contents enables preparation of competent HVJ-E, and makes it possible to rapidly include various types of content in HVJ-E. For example, if a 96-well plate is used, transfection with 96 different types of content can be completed in about 30 minutes. This technique is suitable when high throughput is required, i.e., cases in which multiple genes, siRNAs, etc., need to be treated rapidly on the same plate for analysis of cell function or exploration of new genes.

If plates of other sizes are used, transfection with this product is possible by adjusting the dose level on the basis of well area ratio.

6-1: Transfer of plasmid DNA

- DNA/TE solution concentration: 0.1-0.25 μg/μL
- Cell density: 0.2-1.0 × 10^4 cells/0.1 ml of medium/well of 96-well plate
- Protocol (Method M)

The protocol shown below pertains to the use of 96-well plates.

* This method assumes transfection of adhesive cells.

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (with a 96-well plate) keep 0-8 °C	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 6.25AU (250 μL)
	(2)	Combination with Reagent B and agitation[46] (tapping)	Reagent B: 25 μL
	(3)	Centrifugation at 10,000 g (10,000-12,000 rpm) ant 4°C for 10 minutes. Supernatant is discarded[47]	
	(4)	Sediment suspended in buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[48]	Buffer: 500 μL
	(5)	Suspension prepared in Step 4 is applied to 96-well plate[49] for vector preparation.	5 μL/well
	(6)	DNA/TE solution is added to each well and agitated (with a plate shaker, etc.)	5 μL/well
	(7)	Left to stand for 5 minutes	
Introduction	(8)	Reagent C, diluted 1:16 (Reagent C 35 μL + buffer 525 μL), is added and agitated (with a plate shaker, etc.)	Diluted Reagent C: 5 μL/well
	(9)	Medium is added to each well and agitated (with a plate shaker, etc.)	Medium: 50 μL/well
	(10)	HVJ-E vector suspension added to each well of another 96-well plate containing preincubated cells, and incubated at 37°C under 5% CO_2 (medium renewed as needed).[50]	Suspension (Steps (5) + (6) + (8) + (9)): 65 μL/well
	(11)	Incubation at 37°C under 5%CO_2	

Steps (1) through (4) should be performed on ice.

[46] The amount of Reagent B added should equal 1/10 of the volume before addition (Step (1)).

[47] With this technique, the HVJ-E pellets obtained after centrifugation are likely to collapse. Care is needed to avoid aspirating the pellets when discarding the supernatant.

[48] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[49] For the process of incorporation into HVJ-E, a plate other than that for incubation of the cells to be transfected should be used.

[50] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (5) + (6) + (8) + (9)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

6-2 : Transfer of siRNA

- siRNA (oligo-type) concentration: 0.01-0.05 μg/μL
- Cell density: 0.2-1.0 × 10^4 cells/0.1 ml of medium/well of 96-well plate
- Protocol (Method M for siRNA)

The protocol shown below pertains to the use of 96-well plates.

* This method assumes transfection of adhesive cells.

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, other reagents, and tubes should be cooled adequately in an ice bath. The vector is then prepared in the following steps.

		Step (with a 96-well plate) keep 0-8 °C	Amount of reagent
Incorporation	(1)	HVJ-E taken into a micro-test tube	HVJ-E: 1.75AU (70 μL)
	(2)	Combination with Reagent B and agitation[51] (tapping)	Reagent B: 7μL
	(3)	Centrifugation at 10,000 g (10,000-12,000 rpm) ant 4°C for 10 minutes. Supernatant is discarded[52]	
	(4)	Sediment suspended in buffer (pipetted 20-30 times or more until the suspension becomes uniformly white)[53]	Buffer: 560 μL
	(5)	Suspension prepared in Step 4 is applied to 96-well plate[54] for vector preparation.	5 μL/well
	(6)	siRNA solution is added to each well and agitated (with a plate shaker, etc.)	5 μL/well
	(7)	Left to stand for 5 minutes	
Introduction	(8)	Reagent C, diluted 1:16 (Reagent C 35 μL + buffer 525 μL), is added and agitated (with a plate shaker, etc.)	Diluted Reagent C: 5 μL/well
	(9)	Medium is added to each well and agitated (with a plate shaker, etc.)	Medium: 50 μL/well
	(10)	HVJ-E vector suspension added to each well of another 96-well plate containing preincubated cells, and incubated at 37°C under 5% CO_2 (medium renewed as needed).[55]	Suspension (Steps (5) + (6) + (8) + (9)): 65 μL/well
	(11)	Incubation at 37°C under 5%CO_2	

Steps (1) through (4) should be performed on ice.

[51] The amount of Reagent B added should equal 1/10 of the volume before addition (Step (1)).

[52] With this technique, the HVJ-E pellets obtained after centrifugation are likely to collapse. Care is needed to avoid aspirating the pellets when discarding the supernatant.

[53] When suspending the sediment in the buffer, the mixture is agitated gently (to avoid bubbling) until the suspension becomes uniformly white. Agitation with a vortex should be avoided since it can cause bubbling. It is advisable to use a pipette for this purpose.

[54] For the process of incorporation into HVJ-E, a plate other than that for incubation of the cells to be transfected should be used.

[55] Usually, the medium does not need to be renewed after addition of HVJ-E vector suspension (Steps (5) + (6) + (8) + (9)). If any sign of cytotoxicity is noted, renew the medium after about 10 minutes to 3 hours of exposure.

Troubleshooting Guide for ***GenomONE-Neo***

Problem	Possible cause
Low transfection efficiency	Loss of binding or fusion activity of HVJ-E. (in vitro, in vivo)
	Low efficiency of binding of HVJ-E with target cell membrane. (in vitro)
	Low efficiency of incorporation of nucleic acids into HVJ-E. (in vitro, in vivo)
	Nucleic acids of poor quality. (in vitro, in vivo)
	Cell density is not adequate. (in vitro)
	Molecular size of content is too small (Mw.< 1kDa)or too large. (in vitro, in vivo)
High cytotoxicity (In vitro)	Excessive exposure of cells to HVJ-E vector.
	Plasmid DNA preparation contaminated with large amount of endotoxin.
	Excessive exposure of cells to Reagent C.
	Conditions of cultured cells are not suitable for transfection.
	If above checks or tests prove negative and do not result in any improvement, HVJ-E vector may be extremely cytotoxic to your specific cell type.

NOTE

Manufacturer

ISHIHARA SANGYO KAISHA, LTD.

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

URL: https://www.iskweb.co.jp/eng/products/hvj-e/