

*For Laboratory Research Use Only  
Not for Use in Diagnostic or Therapeutic Procedures*

HVJ Envelope Cell Fusion Kit

***GenomONE™-CF EX***

# **Instruction Manual**

(Ver 1.04)

**!** 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.

## **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. Cell fusion experiments that produce recombinant organisms, etc. may fall within the restrictions of the Cartagena Protocol on Biosafety. Experimenters using this product to produce recombinant organisms must take appropriate measures to prevent diffusion of such organisms before conducting experiments with this product.
3. 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.
4. 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.
5. 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.
6. 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.).
7. 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.
8. 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.
9. 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.).
10. 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.
11. 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.
12. 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.
13. This product is covered by the claims of one or more 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. Specifications

(Cat.# ISK-CF001-EX)

Reagent	Volume
Freeze-dried HVJ-E <sup>1)</sup>	Equivalent to 0.26 mL (1 vial)
HVJ-E suspending buffer	0.5 mL (1 vial)
Cell fusion buffer (20X concentrate) <sup>2)</sup>	10 mL (1 vial)

1) HVJ-E: HVJ Envelope

Moisture can reduce activity. Store the product in a refrigerator, sealed in an aluminum package.

2) Before use, dilute 1:20 with water (e.g.: endotoxin-free water for injection)

## 2. Frequency of use

- For fusion of cells of the same or different types: About 100 runs
- For preparing B cell hybridoma: About 10 runs

## 3. Stability during storage

- Quality assured period of freeze-dried HVJ-E : See the aluminum package for HVJ-E.
- HVJ-E suspension prepared with HVJ-E suspending buffer: For continuous use, store in a refrigerator (2-8°C) for up to two weeks. For extended storage, freeze in working aliquots at -80°C for up to 3 months. Thawing after freezing is possible only once.
- “HVJ-E suspending buffer” and “Cell fusion Buffer” should be stored in a refrigerator (2-8°C).

## 4. Quality

- HVJ-E is a purified product prepared through complete inactivation\* of the genomic RNA of HVJ (Sendai virus). It has neither infective nor proliferative potentials in humans or animals. Inactivation of HVJ has been confirmed for each lot by the viral proliferative potential rule-out test, using cultured cells and fertilized chicken eggs.

\* 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.).

- HVJ-E has been confirmed by sterility test to be free of bacterial and fungal contamination.

! 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).

## 5. Procedures for use

### 5-1: Preparing reagents

- ① Preparing an HVJ-E suspension  
Combine freeze-dried HVJ-E with 0.26 mL ice-cooled “HVJ-E suspending buffer”. Gently pipette the mixture, taking care to avoid bubble formation, to yield a homogeneous suspension. Ice-cool the suspension immediately to avoid reductions in activity.
- ② Preparing “Cell fusion buffer”  
Dilute the “Cell fusion buffer (20X concentrate)” 1:20 with sterile pure water (e.g., endotoxin-free water for injection). The diluted buffer may be stored in a refrigerator.

### 5-2: Procedure for cell fusion

- ① **Suspension Method**  
Use this method when fusing suspension cells of the same or different types.
- ② **Plating Method**  
Use this method when cells adhering to a plate are to be fused to suspension cells.
- ③ **Protocol for preparing B cell hybridoma**  
Use this method when preparing monoclonal antibody-producing cells.

## ① Suspension Method

Step	Operation (Steps 1 through 3 are taken on ice)
1	Suspend cells A and B separately in ice-cooled cell fusion buffer (one-fold concentrate*) using 2 mL tubes (preferably round-bottom tubes) at a concentration of $2 \times 10^5$ cells/25 $\mu$ L. Then mix by pipetting.
2	Add ice-cooled HVJ-E suspension (2.5 $\mu$ L)** to the cells, and mix by tapping.
3	Leave the mixture to stand on ice for 5 minutes <to allow HVJ-E to be adsorbed on the cell surface>***
4	Incubate at 37°C for 15 minutes (mixing by tapping every 5 minutes)**** <to induce cell fusion>
5	Add the medium for cell proliferation, then agitate and transfer to 6-well plate for further incubation (centrifuge removal of HVJ-E is required if necessary)

- \* Prepared by 1:20 dilution of the 20X concentrate cell fusion buffer (included with the kit) with pure water.
- \*\* Adjust the volume of HVJ-E in the range of 0.5-10  $\mu$  L depending on the efficiency of fusion and the degree of cytotoxicity.
- \*\*\* The efficiency of fusion may be elevated if centrifuging (4°C, 2000 rpm, 5 minutes) after Step 3. If centrifuged, the cells should not be re-suspended but should be incubated in the form of a pellet in Step 4. In the case of cells with a higher potential for fusion, centrifuging tends to increase polynucleated cells.
- \*\*\*\* When induction of polynucleated cells or cytotoxicity is excessive, remove the supernatant by centrifugation after incubation.

## ② Plating Method

Step	Operation (Steps 1 through 6 are taken on ice)	
	Cell A (suspension/floating)	Cell B (adhering to 6-well plate)
1	Suspend cell A in ice-cooled fusion buffer (one-fold concentrate*) in a concentration of $2 \times 10^5$ cells/25 $\mu$ L.	
2	Add ice-cooled HVJ-E suspension (2.5 $\mu$ L)**, and mix by tapping.	
3	Leave the mixture to stand on ice for 5 minutes <to allow HVJ-E to be adsorbed on cell A surface>	
4	After centrifuging (4°C, 2000 rpm, 5 minutes), remove the supernatant.	
5	Add ice-cooled fusion buffer 1 mL (one-fold concentrate*), and mix by pipetting. Leave to stand on ice.	Remove the supernatant, and wash the cells with ice-cooled fusion buffer (one-fold concentrate*)
6	Add the whole amount of cell A after treatment with HVJ-E to the cell B cell sheet. Mix gently.	
7	Centrifuge the plate (4°C to room temperature, 1000 rpm, 5 minutes)	
8	Incubate at 37°C for 15 minutes <to induce cell fusion>	
9	Remove the supernatant and add the medium for cell proliferation for further incubation.	

\* Prepared by 1:20 dilution of the 20X concentrate cell fusion buffer (included with the kit) with pure water.

\*\* Adjust the volume of HVJ-E in the range of 0.5-10  $\mu$  L depending on the efficiency of fusion and the degree of cytotoxicity. Adjust the buffer volume in proportion to the incubated area of each culture plate or well.

### ! CAUTION

Experimenters using this product to produce recombinant organisms must take appropriate measures to prevent diffusion of such organisms before conducting experiments with this product. Cell fusion experiments that produce recombinant organisms may fall within the restrictions of the Cartagena Protocol on Biosafety.

### ③ Protocol for preparing B cell hybridoma

Step	Operation (Steps 4 through 6 are taken on ice)
1	<p>&lt;Preparing splenocytes&gt; Remove the spleen from the immunized mouse, remove erythrocytes by hemolysis, and suspend in serum-free RPMI medium to yield a cell suspension (about <math>1 \times 10^8</math> cells/mouse)</p> <p>&lt;Preparing myeloma cells&gt; Harvest cells cultured on a dish (about <math>1 \times 10^7</math> cells) and suspend in serum-free RPMI medium.</p>
2	Combine splenocytes with myeloma cells at a ratio of 10:1 (on a cell count basis) in a 50 mL conical tube.
3	Centrifuge (4°C, 1000 rpm, 5 minutes), and remove the supernatant.
4	Add ice-cooled fusion buffer (one-fold concentrate*) (1 mL per $10^8$ splenocytes), and pipette to yield a homogeneous suspension.
5	Add ice-cooled HVJ-E suspension (25 $\mu$ L per 1 mL cell suspension)
6	Leave to stand on ice for 5 minutes <to induce HVJ-E adsorption on cell surface>
7	After centrifuging (4°C, 1000 rpm, 5 minutes) <to induce cell fusion>, carry on to the next step, without removing the supernatant, using the cells in the form of a pellet without re-suspension
8	Incubate at 37°C for 15 minutes (leave to stand without mixing) <to induce cell fusion> Carry on to the next step, without removing the supernatant.
9	Add the medium for cell proliferation**, preheated to 37°C (50 mL per $10^8$ splenocytes) while mixing gently with a pipette to yield a suspension.
10	Inoculate onto a 96-well plate (100 $\mu$ L/well). Using a routine technique, replace the medium with HAT medium the following day and cultivate.

\* Prepared by 1:20 dilution of the 20X concentrate cell fusion buffer (included with the kit) with pure water.

\*\* The use of low-temperature medium can reduce the efficiency of cell fusion. Use a pre-heated medium.

- *One-point advice*

If fusion efficiency (hybridoma positive rate) is low, changing the splenocyte/myeloma cell mixture ratio (10:1 to 1:1) in Step 1 or changing the volume of HVJ-E in the range of 12.5 to 50  $\mu$  L in Step 5 may optimize fusion conditions.

## Troubleshooting Guide for *GenomONE-CF EX*

Problem	Possible cause
Low fusion efficiency	Loss of binding or fusion activity of HVJ-E.
	Inadequate preparation of “Cell fusion buffer”
	Culture medium is too cold
	Presence of serum in the fusion buffer
	Amount of HVJ-E used for fusion is not optimal For “Suspension Method” and “Plating Method (See p 4)”
	Cell-cell contact is weak and fusion efficiency is low. For “Suspension Method (See p 5)”.
	Splenocyte/myeloma cell mixture ratio is inadequate For “Protocol for preparing B cell hybridoma (See p 6)”
	Amount of HVJ-E used for fusion is not optimal For “Protocol for preparing B cell hybridoma (See p 6)”
High cytotoxicity Induction of polynucleated cells	Excessive exposure of cells to HVJ-E vector. For “Suspension Method (See p 5)”.
	If above checks or tests prove negative and do not result in any improvement, HVJ-E may be extremely cytotoxic to your specific cell type.

## FAQ for *GenomONE-CF EX*

	Question
1	What’s GenomONE-CF EX?
2	Characteristics of HVJ-E
3	Principle of cell fusion triggered by HVJ-E
4	Restriction for GenomONE-CF EX use
5	Expiration date
6	Method for inactivation of HVJ
7	Assessment and confirmation of viral inactivation
8	Methods for confirmation of viral inactivation
9	Bio-safety level for laboratory use, Precautions for use
10	Quality assurance
11	License requirement and commercial use
12	Role of each reagent
13	Constituent and concentration of each Reagent
14	Storage of reconstituted HVJ-E suspension
15	Storage of “HVJ-E suspending buffer” and “Cell fusion Buffer (20X)”
16	Particle size of HVJ-E
17	Number of HVJ-E particles
18	Hemagglutination units (HAU) of HVJ-E
19	Frequency of use
20	Published researches using GenomONE

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