
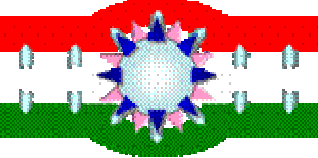
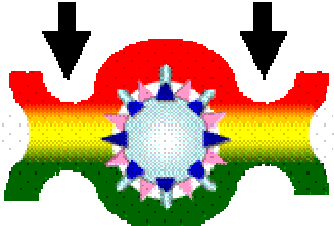


FAQ for *GenomONE-CF EX*

Refer to the GenomONE web page for additional information:

<http://www.iskweb.co.jp/hvj-e/english-default.htm>

	Question	Answer and comment
1	What's GenomONE-CF EX?	GenomONE-CF is a cell fusion kit composed of HVJ Envelope (HVJ-E) and special buffers. It can be used with both adherent cells and suspension cells. Fusion of cells of the same or different types is possible with this kit in only 30 minutes.
2	Characteristics of HVJ-E	<p>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.</p> <p>It is known that the HN protein in the tunica externa (envelope) of the Sendai virus recognizes the receptor (possessing sialic acid at the terminal of sugar chain) on the cell membrane and adsorbs it, leading to the induction of membrane fusion mediated by F protein (another component of the envelope). 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.</p>
3	Principle of cell fusion triggered by HVJ-E	<p>Step 1 and 2.</p> <div style="text-align: center;">  </div> <p>If HVJ-E is added in amounts of more than several hundred HVJ-E per cell at low temperatures (0-8°C), HVJ-E is immediately adsorbed on the cell surface mediated by the receptor (acetyl type sialic acid recognized by HN protein) (Step 1), and cells undergo agglutination cross-linked by HVJ-E particles (Step 2).</p>

		<p style="text-align: center;">Step 2</p>  <p>At this stage, the hydrophobic domain at the N-terminal of cleaved F protein (F1) penetrates into the double lipid layer of the cell membrane, causing distortion of the membrane severe enough to allow an inflow of ions.</p> <p>Step 3.</p> <p style="text-align: center;">Step 3</p>  <p>If this cell/HVJ-E complex is heated at 37°C, the distortion of the cell membrane is further expanded, accompanied by temporary alteration of the cell membrane lining structure. This change is transient and the membrane soon returns to its normal structure. However, if a strong hydrophobic connective force is applied at this stage, fusion between cell membranes takes place (Step 3).</p> <p><u>Reference</u> Okada Y., <i>et al.</i>, <i>Exp. Cell Res.</i>, <u>93</u>, 368-378 (1975).</p>
4	Restriction for GenomONE-CF EX use	GenomONE-CF EX is developed, designed and sold for research purposes only. It is not to be used for human or animal diagnostic or therapy (drug purposes).
5	Expiration date	The period of guarantee of quality of freeze-dried HVJ-E is printed on the aluminum package for HVJ-E.
6	Method for inactivation of HVJ	Although HVJ-E uses HVJ (Sendai virus/ Murine parainfluenza virus 1) as a raw material, the genomic RNA of HVJ has been completely inactivated by drug treatment*. The HVJ-E will not

		<p>proliferate or exhibit pathogenic effects in humans or animals.</p> <p>* Reference:</p> <p>Prior, P. <i>et al.</i>: BioPharm, 22-33 (Oct. 1996)</p> <p>Kaneda, Y. <i>et al.</i>: Advances in Genetics, 53, 308-332 (2005).</p>
7	Assessment and confirmation of viral inactivation	Inactivation of HVJ has been confirmed for each lot by the viral proliferative potential rule-out test, using cultured cells and growing chicken eggs.
8	Methods for confirmation of viral inactivation	<p>Lack of possibility of infection or proliferation of HVJ-E in humans or experimental animals has been confirmed by means of the following three methods.</p> <p>(1) Assay using cultured cells</p> <p>(2) Assay using fertilized chicken eggs</p> <p>(3) Assay using mice</p>
9	<p>Bio-safety level for laboratory use</p> <p>Precautions for use</p>	<p>GenomONE can be used safely in ordinary laboratories. Although HVJ-E uses HVJ (Sendai virus/Murine parainfluenza virus 1) as a raw material, the genomic RNA of HVJ has been completely inactivated by drug treatment. HVJ-E will not proliferate or exhibit pathogenic effects in humans or animals.</p> <p>However, when using this product for 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.</p> <p>Furthermore, 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.</p>
10	Quality assurance	<ul style="list-style-type: none"> ■ 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. ■ Endotoxin level has been confirmed to be less than 2.5

		EU/mL (Limulus Amebocyte lysate gel clot assay).
11	License requirement and commercial use	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.).
12	Role of each reagent	<ul style="list-style-type: none"> ■ Freeze-dried HVJ-E: HVJ-E is a purified vesicle prepared through complete inactivation of HVJ (Sendai virus), in which only the cell membrane-fusing capability of the envelope protein of HVJ is retained. ■ HVJ-E suspending buffer: Neutral buffer of physiological concentration used for reconstituting, suspending or diluting HVJ-E or other purposes. ■ Cell fusion buffer: Optimized buffer used for cell fusion. "Cell fusion buffer (20X concentrate)" should be diluted 1:20 with sterile pure water (e.g., endotoxin-free water for injection) before use.
13	Constituent and concentration of each Reagent	Not disclosed.
14	Storage of reconstituted HVJ-E suspension	<ul style="list-style-type: none"> ■ 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.
15	Storage of "HVJ-E suspending buffer" and "Cell fusion Buffer (20X)"	Stored in a refrigerator (2-8°C). Do not freeze.
16	Particle size of HVJ-E	Approximately 300 nm (200-400 nm)
17	Number of HVJ-E particles	40μL of reconstituted HVJ-E suspension includes approximately 10^9 - 10^{10} particles of HVJ-E.
18	Hemagglutination units (HAU) of HVJ-E	40μL of reconstituted HVJ-E suspension equivalent to approximately 1,000-2,000 hemagglutination units (HAU).
19	Frequency of use	<ul style="list-style-type: none"> ■ For fusion of cells of the same or different types: About 100 runs ■ For preparing B cell hybridoma: About 10 runs



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