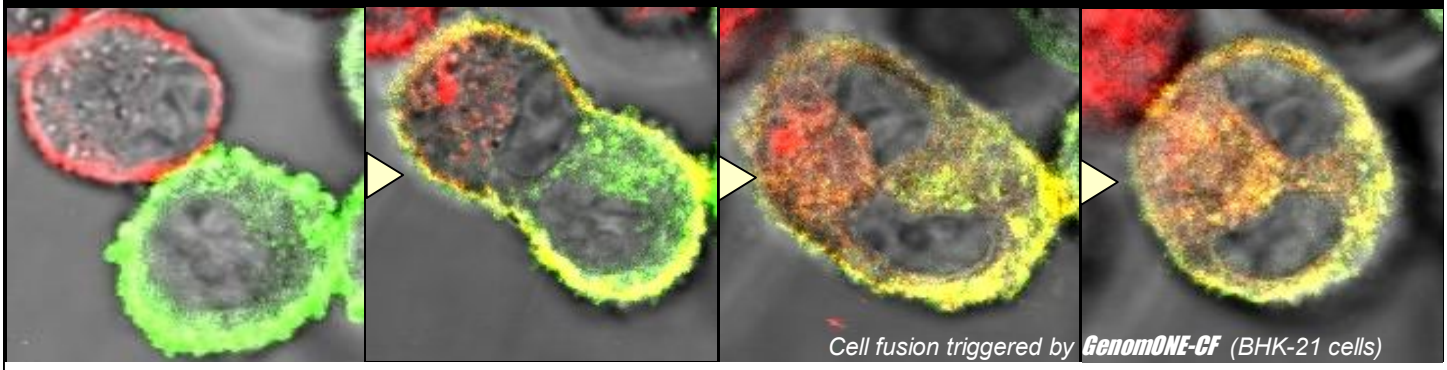
For research use

Ver.2.5ENG (ISK10AU02)

HVJ Envelope Cell Fusion Kit

GenomONE™-CF EX

Data Sheet



Research on cancer vaccinations and cancer immunology
Fusion of cancer cells and dendritic cells, *etc.*

Preparation of monoclonal antibodies
Fusion of antigen-sensitized B cells and myeloma cells

Other
Cell fusion involving PEG and electrofusion

Research on embryonic development/differentiation
Nuclear transfer (nuclear replacement)

Research on regenerative medicine and cytotherapy
Fusion of somatic cells and stem cells, *etc.*

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GenomONE™-CF Ex Overview

Scope of application

GenomONE-CF Ex is a cell fusion kit composed of HVJ Envelope (HVJ-E) and special buffers. It can be used with both adhering cells and floating cells. Fusion of cells of the same or different types is possible with this kit in only 30 minutes.

This kit resolves the following problems,
encountered when attempting cell fusion using polyethylene glycol (PEG) or electrofusion

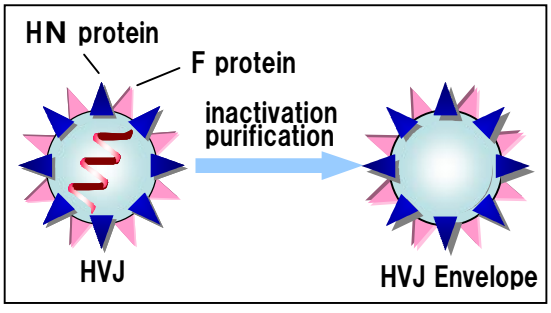
- Operation is too complex
- Toxicity is high and the efficiency of cell fusion low
- Reproducibility is low
- Much time is needed to optimize fusion conditions
- Rapid or simultaneous processing of many samples is not possible due to the necessity for strict control of reactions

etc.

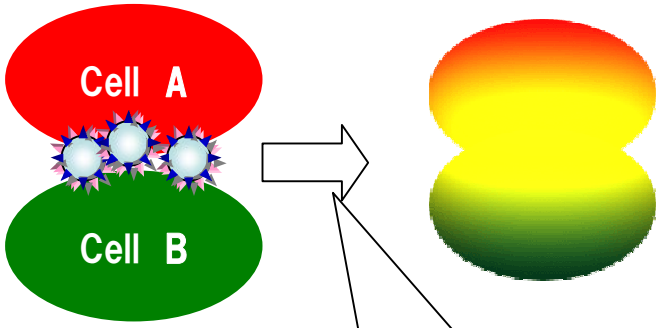
What is HVJ Envelope (HVJ-E)?

HVJ Envelope is a purified product prepared through complete inactivation of Sendai virus (HVJ*). It is a vesicle in which only the cell membrane-fusing capability of the envelope protein of Sendai virus is retained.
It is known that the HN protein in the tunica externa 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.

***HVJ : Hemagglutinating Virus of Japan**

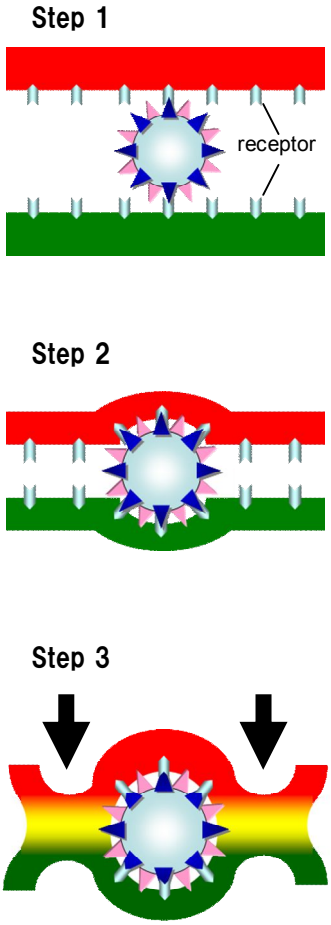


Principle for cell fusion triggered by HVJ-E



Ⅰ.
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).
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.

Ⅱ.
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).

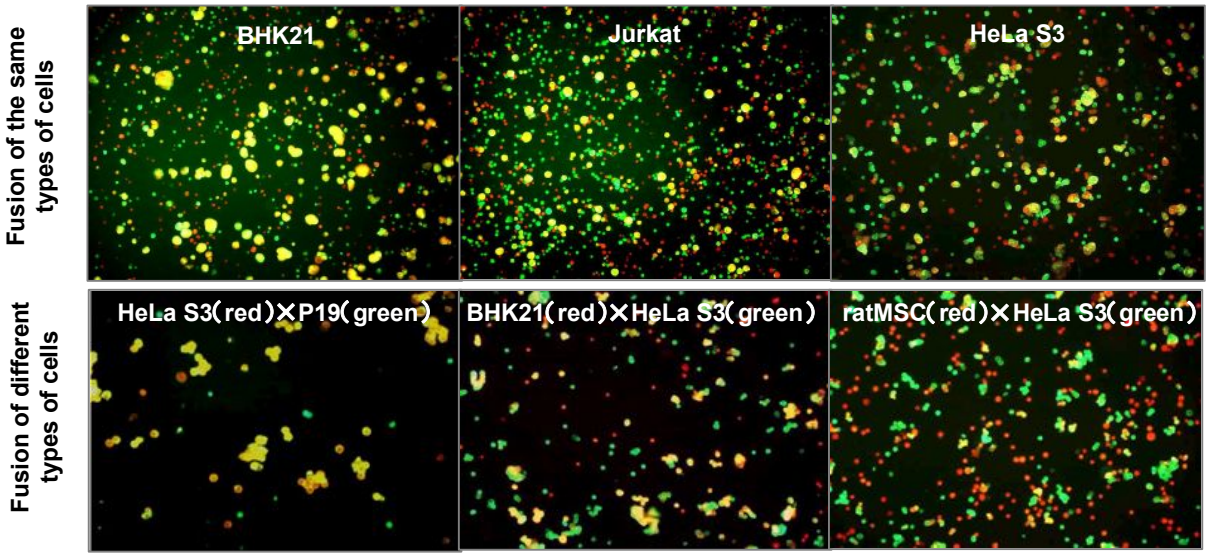


The figure illustrates a local model of two cells cross-linked by one HVJ-E particle.

[Reference]
Okada Y., *et al.*, *Exp. Cell Res.*, 93, 368-378 (1975).
Okada, Y., Cell Fusion and Life Science, Brain Center (2001).

Examples of cell fusion experiments

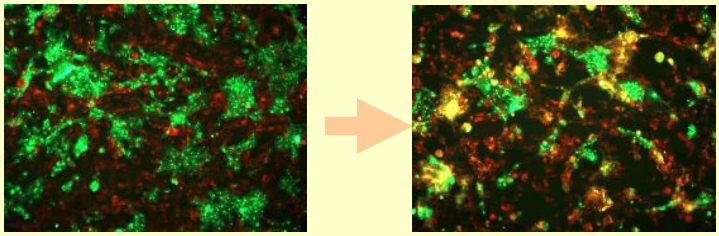
(1) Fusion of suspension cells



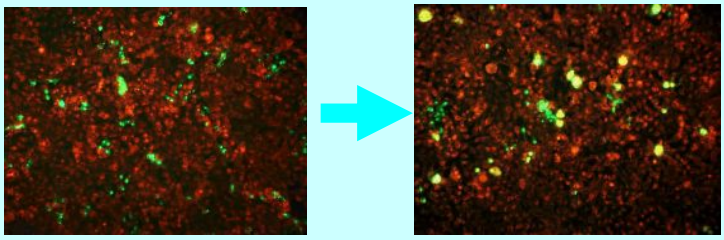
Cells labeled with red fluorescence*1 were combined with cells labeled with green fluorescence*2 (each 1-2 x10^5 cells) in a fusion buffer (50 μ L). HVJ-E was added to the mixture, followed by incubation on ice for 5 minutes and further incubation at 37°C for 15 minutes. Fused cells (yellow) were obtained.

(2) Fusion of adherent cells and suspension cells

Fusion of adhering BHK-21 cells (red) and floating P19 cells (green)

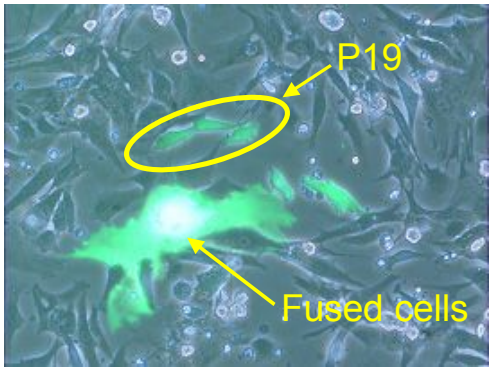


Fusion of adhering HeLa S3 cells (red) and floating P19 cells (green)



Cells labeled with red fluorescence*1 adhering to a 12-well plate were combined with 1 x 10^5 suspension cells labeled with green fluorescence*2 and pre-treated with HVJ-E (suspended in fusion buffer). Each plate was centrifuged at 1000 rpm at room temperature for 5 minutes. This was followed by incubation at 37°C for 15 minutes. The medium was then replaced with a medium for cell proliferation, and the cells were incubated further. Fused cells (yellow) were observed 3 hours later.

Fusion of adhering neonatal rat primary cardiac myocytes and floating P19 cells (GFP-expressed cells)



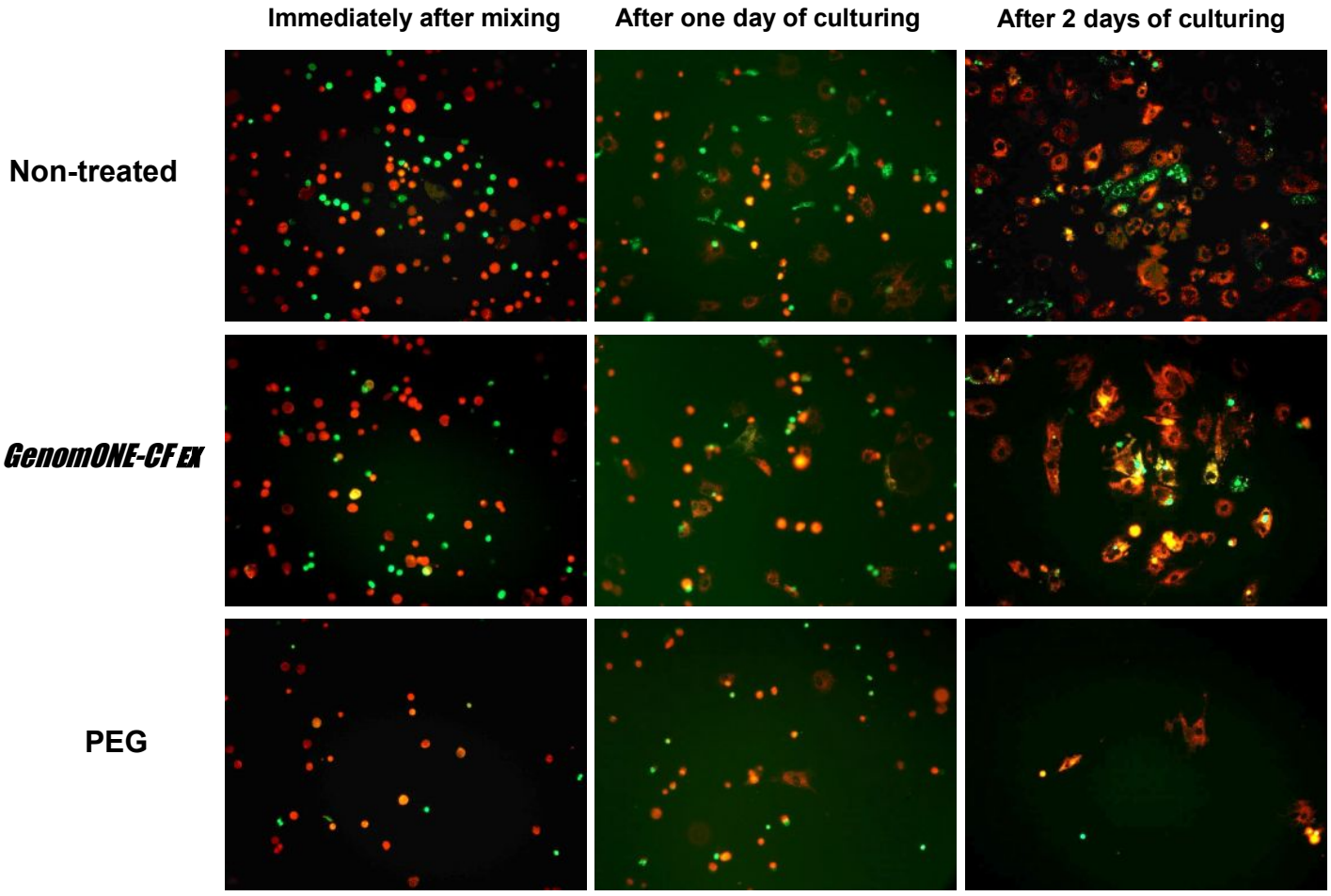
2.5 x 10^5 P19 cells pretreated with HVJ-E (emitting green fluorescence due to GFP gene transfection) were added to rat primary cardiac myocytes adhering to a 12-well plate. Each plate was centrifuged at 1000 rpm at 20°C for 3 minutes. The medium was then replaced with a medium for cell proliferation, and the cells incubated further. Twenty-four hours later, cells showing strong GFP expression due to fusion with P19 cells were found among the pulsatile myocytes in the HVJ-E treated wells.

Data supplied by Dr. Hironori Nakagami,
Division of Gene Therapy Science, Graduate School of Medicine,
Osaka University, Japan.

*1: For fluorescent labeling, PHK26 Red Fluorescent Cell Linker Kit (Sigma) was used.
*2: For fluorescent labeling, PKH67 Green Fluorescent Cell Linker Kit (Sigma) was used.

Example comparisons with PEG method

(1) Comparison with PEG method in the fusion of different types of cell



Rat MSC cells (rat bone marrow-derived mesenchymal stem cells) labeled with red fluorescence[*1] were combined with rat primary cardiac myocytes labeled with green fluorescence[*2] (each 2 x 10^5 cells) in 50 μ L of a buffer for cell fusion. The mixture was incubated on ice for 5 minutes and then at 37°C for 15 minutes. As a result, fused cells (yellow) were formed (GenomONE-CF suspension method). Fused cells adhering to the plate were also observed after 1-2 days of culturing. In the PEG-treated group, high cytotoxicity appeared immediately after cell fusion, reducing the number of fused cells obtained.

(*1) Labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma)
(*2) Labeled with PKH67 Green Fluorescent Cell Linker Kit (Sigma)

(2) Comparison with PEG method in hybridoma preparation

	Hybridoma positive rate	ELISA positive rate
HVJ-E	100%	96%
PEG	40%	30%

The footpad of mice was immunized with KLH three times (on Day 1, 4 and 7). Three days after the last immunization (Day 10), lymphocytes were collected from the lymph nodes of these mice. These lymphocytes were combined with myeloma cells and the mixture treated with HVJ-E or PEG (molecular weight: 3,000-3,700) to induce cell fusion. Twenty-eight days later, proliferation of hybridomas was checked and wells showing formation of KLH-specific antibody were counted (ELISA).

Data supplied by Dr. Masahiro Tomita, Laboratory of Molecular Bioengineering, Department of Chemistry for Materials, Faculty of Engineering, Mie University, Japan.

Footnote: Depending on the type of antigens or myeloma cells used, the conditions for fusion shown in the above examples may require some optimization.

Example comparisons with PEG method

(3) Comparison with PEG method in hybridoma preparation

【Procedure】

Normal BALB/c mouse splenocytes (1×10^8cells) not sensitized with antigen were fused to X63-Ag8.653 myeloma cells($1X10^7$cells) using ***GenomONE -CF EX*** or PEG1500 (50mL of fused cell suspension was obtained). One-fifth (10mL) of the suspension obtained with each agent was inoculated onto a 96-well plate (Day 0). Beginning the following day, half of the culture medium (10%FBS/RPMI1640) was replaced with HAT medium at five points of time(Days 1, 2, 3, 5, and 8), and the growth of colonies in each well was assessed on Days 10 - 11 to determine the hybridoma-positive rate(an indicator of efficiency of fusion). On Day 12, mouse antibody level (IgG + IgA + IgM) in the supernatant was measured by ELISA,to calculate the antibody production-positive rate. The effect of adding a commercially available hybridoma supplement to the medium after fusion was also assessed (supplement was also added to the HAT medium).

【Results】

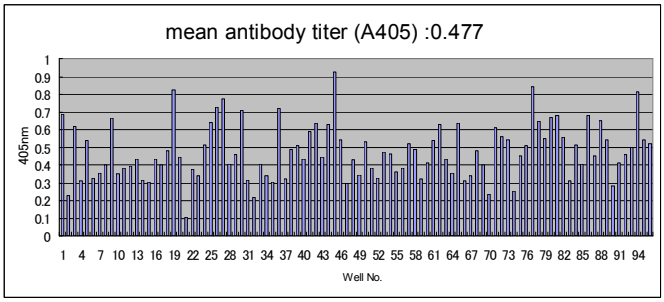
Hybridoma-positive rate (efficiency of cell fusion) and antibody (mouse Ig) production-positive rate

	Hybridoma supplement	Hybridoma-positive rate	Antibody production-positive rate
GenomONE -CF EX	−	38/96 (40%)	9/96 (9%)
	+	96/96 (100%)	96/96 (100%)
PEG1500	−	3/96 (3%)	1/96 (1%)
	+	36/96 (38%)	9/96 (9%)

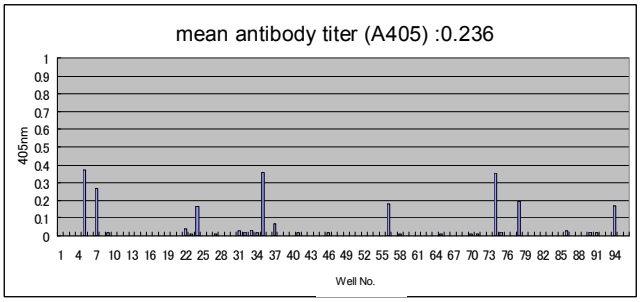
Use of ***GenomONE-CF EX*** resulted in more efficient formation of antibody-producing hybridoma than that of PEG. The efficiency of cell fusion mediated by ***GenomONE-CF EX*** was increased by the addition of hybridoma supplement to the medium used for incubation after cell fusion.

Antibody production

GenomONE-CF EX **/ with supplement**



PEG1500 / with supplement



The vertical axis of this graph indicates the amount of antibody produced.
The wells were considered antibody (mouse Ig) production-positive if A405 absorbance was ≧0.05.

When the cells fused using ***GenomONE-CF EX*** were incubated in medium containing a supplement, colony growth improved and the amount of antibody produced per antibody-positive well increased, resulting in a markedly greater mean amount of antibody produced, compared to the method of cell fusion using PEG.

The results shown above are an example, and experimental conditions need to be optimized depending on the type of antigen, myeloma cell, supplement, *etc.* used.

Example of rapid preparation of monoclonal antibody

An example is presented of an experiment designed to rapidly prepare antibody- producing hybridoma using mouse spleen cells immunized only once

Antigen prepared

Antigen: CML [N$^{\varepsilon}$-(carboxymethyl) lysine]- BSA [bovine serum albumin] conjugate.

Immunized once

(2 weeks)

Antigen (CML-BSA conjugate), 0.1 mg, injected intracutaneously together with CFA (Adjuvant; Sigma F-5881) into the base of the tail of mouse, followed 2 weeks later (without final immunization) by removal of the spleen from the mouse.

Cells fusion

(10 days)

Fusion of removed spleen cells (6×10^7cells) to mouse myeloma (X-63-Ag8.653, 1.2×10^7cells) using ***GenomONE-CF Ex***.

Checking hybridoma proliferation

After fusion, the cells are suspended in 30 mL of culture medium (10%FBS/RPM1640). A 15 mL portion of the mixture is combined with a commercial supplement, (HLCM: Cell Science & Technology Institute, Inc. # 0232, final 0.5-2%) followed by inoculation onto three 96-well plates. From the following day on, half of the medium is renewed daily with HAT medium. HLCM is added in the same fashion at each renewal of medium. Ten days later, colony formation and supernatant antibody titer are evaluated (ELISA).

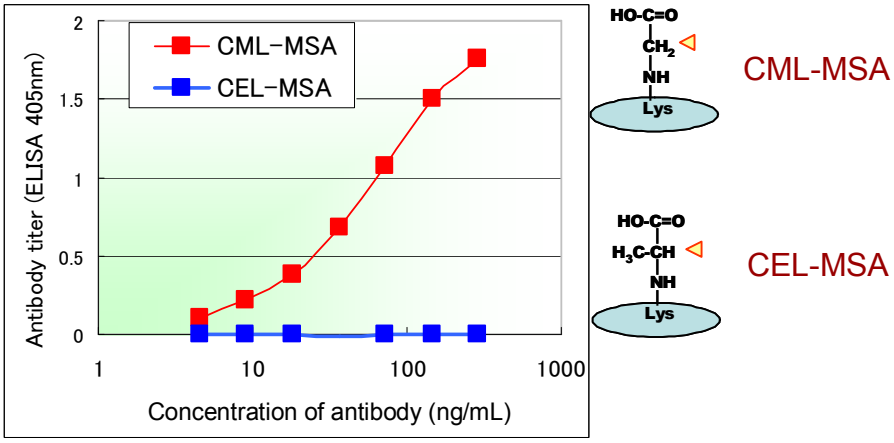
Cloning

Number of hybridoma-positive wells (positive rate, %)	Number of CML antibody production-positive wells (positive rate, %)
285 / 288 (99 %)	9 / 288 (3.1%)

Specificity selected

After a single session of cloning, a CML specific antibody-producing cell line 43-2C is established from the hybridomas with diverse patterns of specificity (compared with CEL-binding potential).

Rapid acquisition of CML-specific antibody !



Specificity of 43-2C (IgG_1, κ) antibody

CML: N$^{\varepsilon}$-(carboxymethyl) lysine.
One of the major components of Maillard reaction products (AGE).
Acquisition of antibody is reported to be difficult with the conventional method involving fusion by PEG after 4 sessions of immunization.

CEL: N$^{\varepsilon}$-(carboxyethyl) lysine.
A major component of AGE, resembling CML.

MSA : mouse serum albumin

The results shown above are an example.
Optimization of experimental conditions depending on the type of antigen, myeloma, supplement, *etc.* is needed.

Published researches using HVJ-E

No.	Reference	Fused cell	Application
1	***Journal of Immunology***, 173, 4297-4307 (2004).	Dendritic cells and B16BL6 melanoma cells	Cancer immunology
2	***Mutation Research***, 562, 11-17 (2004).	Nornal human fibroblasts (G0/G1) and HeLa (M phase)	Premature chromosome condensation (PCC)
3	***Radiation Research***, 165, 59-67 (2006).	Nornal human fibroblasts (G0/G1) and HeLa (M phase)	Premature chromosome condensation (PCC)
4	***Chromosome Science***, 9, 65-73 (2006).	Microcell and HT1080 Microcell and hiMSC (immortalized human mesencymal stem cell)	Microcell mediated chromosome transfer
5	***Hybridoma (Larchmt)***, 26, 381-385 (2007).	Rat lymph node cells (immunized) and mouse myeloma cells (P3U1)	Preparation of monoclonal [Immunogen: recombinant TARSH-CH protein]
6	***Immunology letters,*** 121, 97-104 (2008).	Rat lymph node cells (immunized) and mouse myeloma cells (P3U1)	Preparation of monoclonal antibodies
7	***Human Reproduction,*** 23, 1377-1384 (2008).	Mouse enucleated oocyte and nucleus	Nuclear transfer
8	***Biosystems,*** 92, 226-232 (2008).	Rat cardiac myocute and Rat cardiac fibroblast	Analysis of cellular function
9	***Journal of Cell Science***,122, 912- 918 (2009).	HeLa	Analysis of cellular function
10	***International journal of Oncology***, 35, 249-255 (2009).	Dendritic cells and Meth A cells (BALB/c-derived fibrosarcoma)	Cancer immunology
11	***Nature,*** 461, 367-372 (2009).	Macaca mulatta enucleated oocyte and nucleus	Mitochondrial gene replacement
12	***Reproduction***, 137(4), 625-632 (2009).	Mouse enucleated oocyte and nucleus	Nuclear transfer
13	***Biochem. Biophys. Res. Commun.,*** 390, 547-551 (2009).	Mouse spleen cells (immunized) and mouse myeloma cells (P3U1)	Preparation of monoclonal antibodies [Immunogen: synthetic human IDH1R132H peptide]
14	***Biochem. Biophys. Res. Commun***., 391, 750-755 (2010).	Mouse spleen cells (immunized) and mouse myeloma cells (P3U1)	Preparation of monoclonal antibodies [Immunogen: lacto-series gangliosides 30-isoLM1 and 30,60-isoLD1]
15	***Stem cells and development*** , 19(2), 229-238 (2010).	Mouse adult bone marrow mononuclear cells and Mouse ES cells / Mouse embryonic fibroblasts and Mouse ES cells	Reprogramming of somatic cells
16	***Biochem. Biophys. Res. Commun***., 394, 1053-1057 (2010).	Mouse embryonic fibroblasts and Mouse ES cells	Reprogramming of somatic cells
17	***Nature***, 465, 82–85 (2010).	Human zygotes	Pronuclear transfer
18	***Nature Protocols***, 5(6), 1138-1147 (2010).	Macaca mulatta enucleated oocyte and nucleus	Mitochondrial gene replacement
19	***Hybridoma (Larchmt),*** 29(1), 7-11 (2010).	Rat lymphoid cells(immunized) and Mouse myeloma cells P3U1	Preparation of monoclonal antibodies
20	***Stem Cells*** <in press>	Human Es cells and Human fibroblasts	Reprogramming of somatic cells

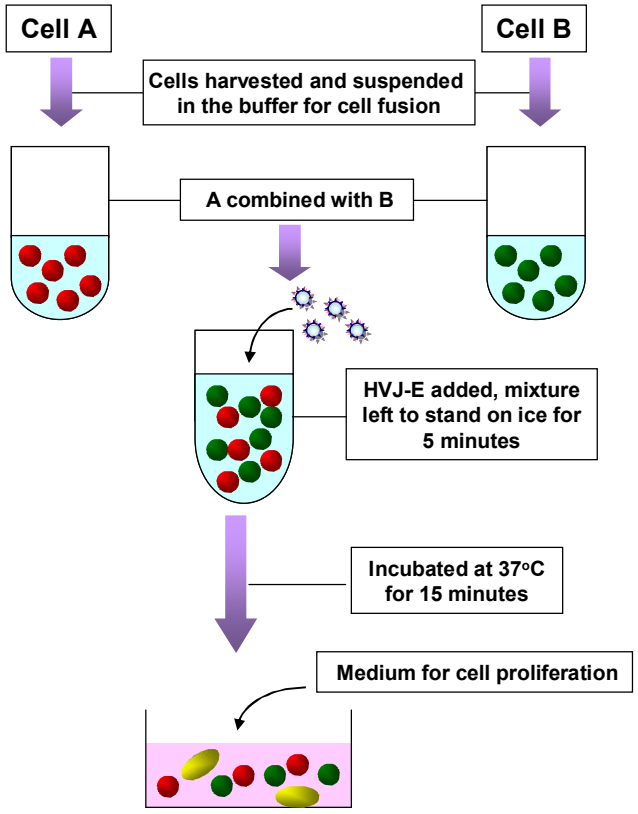
Product Overview

Component	Volume/Container	Storage	Cat. #
Freeze-dried HVJ-E	Equivalent to 0.26 mL/tube	Refrigerated (2-8°C)	ISK-CF001EX
HVJ-E suspending buffer	0.5mL/tube		
Buffer for cell fusion (20x concentrate)	10mL/tube		

Procedure

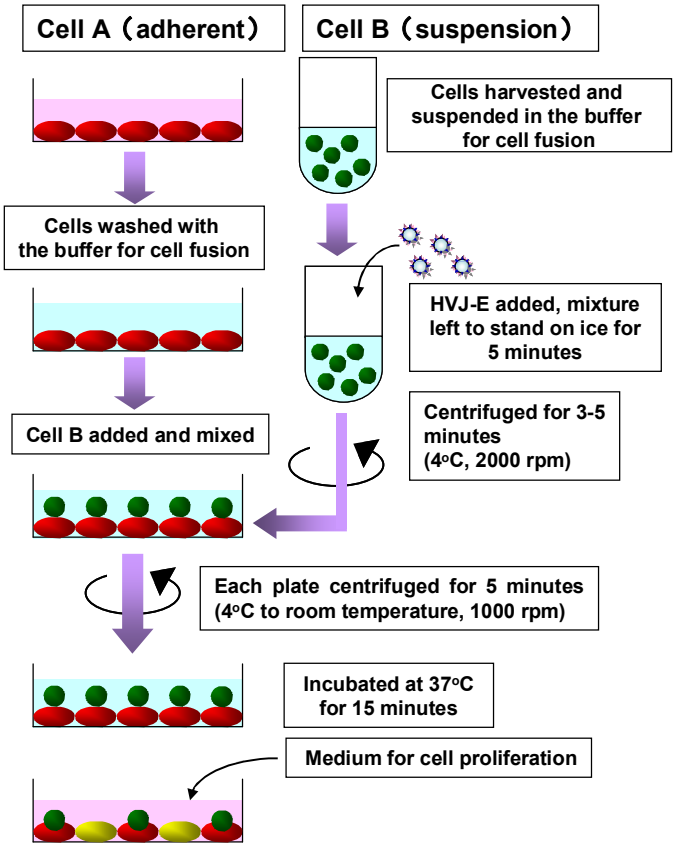
① Suspension Method

The suspension method is used to fuse suspension cells with suspension cells of the same or different type.



② Plating Method

The plating method is used to fuse adherent cells with suspension cells.



Frequency of use (example)

Fusion of cells of the same or different types: about 100 runs
Preparing B cell hybridoma: about 10 runs

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