

GenomONE™ - CF EX

An experimental method designed to fuse donor cells to mouse enucleated oocytes using inactivated Sendai virus (HVJ Envelope: HVJ-E)

[Preparation]

HVJ-E suspension

Suspend freeze-dried HVJ-E in 0.2mL of ice-cooled HVJ-E suspension by pipetting. Infuse the HVJ-E suspension at 5 μ L/tube, and store at -80°C. Thawing of the frozen HVJ-E is possible only once. (ice-cooling)

Cell fusion buffer

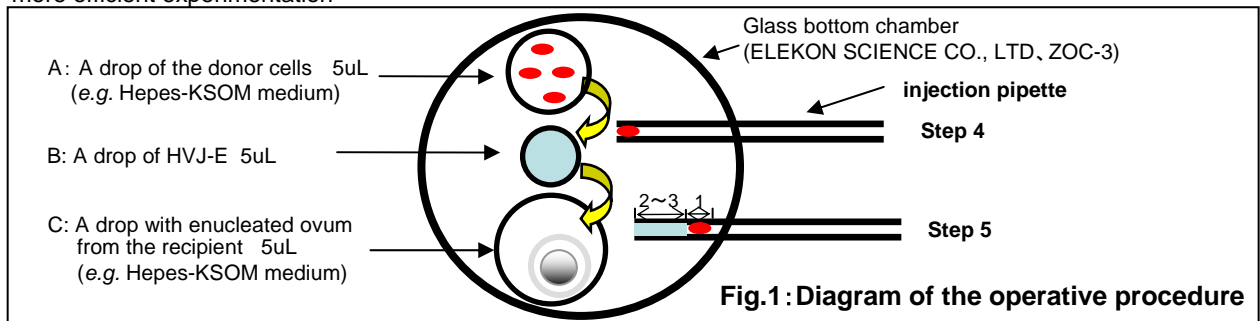
Dilute required amounts of 20X cell fusion buffer for 20-fold with sterile purified water store the diluted buffer refrigerated after dilution.

[Nuclear transplantation protocol: (cumulus cells as nuclear as donors*)]

Step1: Thaw the frozen HVJ-E suspension on ice (and store on ice until use)

Step2: With the prepared cell fusion buffer, dilute the HVJ-E suspension according to type of donor cells (prepare before use; see reverse side for dilution ratio)

Step3: Place each drop of the donor cells, HVJ-E, and enucleated recipient oocytes at room temperature(Fig.1).Until you become accustomed to the nuclear transplantation procedure, use of as few as 5 to 10 enucleated oocytes into the drops enables more efficient experimentation



Step4: Aspirate a donor cell from drop A with an injection pipette

Step5: Aspirate HVJ-E from drop B. The volume of HVJ-E should be 2-3 times of the donor cell.

Step6: Transplant the donor cell with HVJ-E into the enucleated oocyte in drop C, so as to press donor cells and HVJ-E together (see Fig.2)

Step7: Culture in a 5% CO₂ incubator at 37°C for 15 to 30 minutes (Fig.3)

Step8: Verify fusion (Fig.4)

Step9: Transfer the oocyte to the medium for stimulation (strontium and cytochalasin B-containing Ca²⁺, Mg²⁺- free KSOM medium) and culture for 5 to 6 hours. Following verification of pronuclear formation, transfer to the KSOM medium and culture up to the targeted stage of development

Step 6

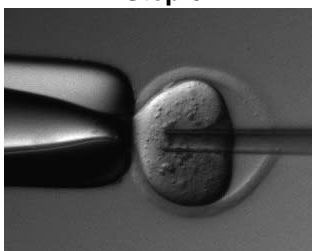


Fig.2: Transfer of the donor cell to an enucleated oocyte

Step 7

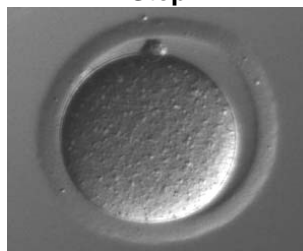


Fig.3: Immediately after transplantation (the donor cell has adsorbed to the top of the enucleated oocyte)

Step 8

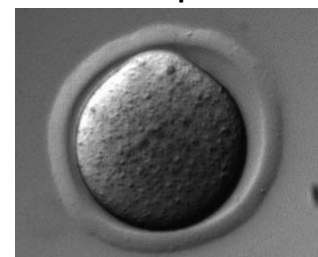


Fig.4: Fifteen to 30 minutes after transplantation (fusion of the donor cell and the enucleated oocyte by HVJ-E has been completed)

Data : Dr. Nami Motosugi and Dr. Minoru Kimura

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※[Case1] Embryonic Stem cells (ES cells) as nuclear donors: Follow procedures from step1 to step9 as in the case of cumulus cells.

[Case2] Preovulatory oocytes (GV oocytes) or fertilized egg (pronuclear) as nuclear donors: In Step9, transfer the ovum to the KSOM medium and culture up to the targeted stage of development.

【Result】

HVJ-E dilution ratio and fusion efficiency in mouse nuclear transplant experiments

| Type of donor cell | HVJ-E dilution ratio * | Fusion efficiency ** |
|---------------------------------|------------------------|----------------------|
| Cumulus cell | 5 | 70 ~ 80 % |
| Embryonic stem cell (ES cell) | | 80 ~ 90 % |
| Preovulatory oocyte (GV oocyte) | 10 | 90 ~ 100 % |
| Fertilized egg (pronuclear) | | 100 % |

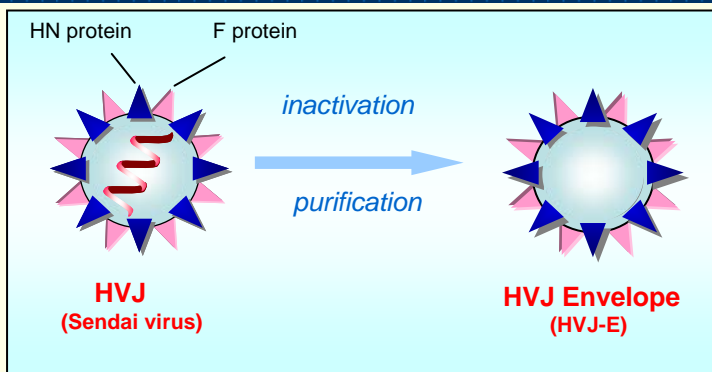
* Diluent of HVJ-E suspension and buffer are prepared before use

** $n \geq 200 \sim 300$

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■ What is HVJ Envelope (HVJ-E) ?



HVJ Envelope is a purified product prepared through complete inactivation of Sendai virus (HVJ: Hemagglutinating virus of Japan). It is a vesicle in which only the cell membrane-fusing capability of the envelope protein of Sendai virus is retained. The genomic RNA of the Sendai virus contained in HVJ-E has been inactivated completely and has neither infective nor proliferative potentials in humans or experimental animals. HVJ-E can be used safely at ordinary laboratories, without requiring any special operations or facilities.

Kaneda, Y., *et al.*: Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system.

Molecular Therapy, 6, 219-226 (2002)

Specifications of *GenomONE-CF EX*

| Cat. # | Freeze-dried HVJ-E (Equivalent to 0.26 mL/vial) | HVJ-E suspending buffer (0.5 mL/vial) | 20× Cell fusion buffer (10 mL/vial) |
|---------|--|--|--|
| CF001EX | 1 | 1 | 1 |

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