

Troubleshooting Guide for *GenomONE-Neo EX*

Refer to the GenomONE web page for additional information:

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

Problem	Possible cause	Suggestions and recommendation
Low transfection efficiency	Loss of binding or fusion activity of HVJ-E (In vitro, in vivo)	<p>Check the condition of preparation and storage HVJ-E.</p> <ul style="list-style-type: none"> ● Because the activity of freeze-dried HVJ-E can be reduced by exposure to high temperature or high relative humidity, refrigerated storage with sealing in an aluminum bag is required. ● Freeze-dried HVJ-E should be reconstituted with ice-cooled Buffer and the suspension should be immediately stored in an ice-cooled bath or in a refrigerator (2-8°C). The HVJ-E suspension gradually loses activity if the temperature is above 8°C. ● After reconstitution, the HVJ-E suspension should be stored in a refrigerator (2-8°C) and <u>should be used within 2 weeks</u>. Since thawing of frozen suspension can reduce transfection activity, <u>reconstituted suspension should not be stored frozen</u>.
	Low efficiency of binding of HVJ-E with target cell membrane (In vitro)	<ul style="list-style-type: none"> ● Increase the amount of Reagent C two- to four-fold compared to the standard amount (please note that Reagent C may have cytotoxic effects in some cell lines). ● Reduce the amount of medium used for transfection and increase the concentrations of HVJ-E vector and Reagent C. ● (For adherent cells) 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). ● (For suspension cells) Extend the duration of centrifugation of the mixture of HVJ-E vector and cells to about 60 minutes.

	Low efficiency of incorporation of nucleic acids into HVJ-E (In vitro, in vivo)	<ul style="list-style-type: none"> ● Check the incorporation procedure. The amount of Reagent B added should equal to 1/10 of the fluid volume. Excessive addition of Reagent B could result in degradation of membrane function of HVJ-E. ● Increase the concentration of nucleic acid used for incorporation into HVJ-E two- to four-fold compared to the standard.
	Nucleic acids of poor quality (In vitro, in vivo)	<ul style="list-style-type: none"> ● Check the purity of nucleic acids. Plasmid DNA used for transfection should be of high quality. ● Endotoxin level should also be reduced using appropriate purification tools, since endotoxins are reported to be cytotoxic to some cell lines (Huh-7 etc.) and some primary cultured cells.
	Cell density is not adequate (In vitro)	<ul style="list-style-type: none"> ● Number of target cells may be inadequate. Use adherent cells at 50-80% confluency.
	Molecular size of content is too small (Mw.<1kD) or too large (In vitro, in vivo)	<ul style="list-style-type: none"> ● Using the standard incorporation procedure, molecules with molecular weights less than 1kDa could not be trapped in HVJ-E and might leak through HVJ-E membrane. ● In contrast, the possibility of incorporating plasmid DNAs or proteins larger than 15kbp (DNA) or 150kDa (IgG), respectively, has yet to be clearly determined.
	Serum and antibiotics (In vitro)	<ul style="list-style-type: none"> ● Usually, addition of serum and antibiotics to the transfection medium (introduction step) does not affect transfection efficiencies. However, reduction of concentrations of serum or antibiotics might increase transfection efficiency.
High cytotoxicity (In vitro)	Excessive exposure of cells to HVJ-E vector	<ul style="list-style-type: none"> ● Reduce the amount of HVJ-E used or the amount of HVJ-E vector added to the medium. ● (For adherent cells) Wash the HVE-J vector 10 minutes to 3 hours after addition to the cells, and replace the medium. ● (For suspension cells) 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.
	Plasmid DNA preparation contaminated with large amount of endotoxin	<ul style="list-style-type: none"> ● Endotoxin level should also be reduced using appropriate purification tools.
	Excessive exposure of cells to Reagent C	<ul style="list-style-type: none"> ● Reduce the amount of Reagent C (1/2-1/4) or skip use of this reagent.

	Conditions of cultured cells are not suitable for transfection	<ul style="list-style-type: none"> ● Since cells might be contaminated with mycoplasma, check using the appropriate mycoplasma detection tool. Eliminate the mycoplasma or use fresh uncontaminated culture.
	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	<ul style="list-style-type: none"> ● Since HVJ-E particles strongly bind and fuse with target cell membrane within several minutes, cytotoxicity to very sensitive cell lines may difficult to completely eliminated. However, the following steps can be taken to reduce cytotoxicity. ● In the introduction step, binding of HVJ-E vector and cells is performed at 4°C or on ice for 15 min (binding of HVJ-E particles to cell membrane is achieved at even lower temperature), remove the HVJ-E vector thereafter and replace the medium. ● Perform the transfection at a higher cell density.

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