

## Troubleshooting Guide for *GenomONE-CFex*

Refer to the GenomONE web page for additional information:

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

<b>Problem</b>	<b>Possible cause</b>	<b>Suggestions and recommendation</b>
Low fusion efficiency	Loss of binding or fusion activity of HVJ-E.	<p>Check the condition of preparation and storage of HVJ-E.</p> <ul style="list-style-type: none"> <li>● 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.</li> <li>● Freeze-dried HVJ-E should be reconstituted with ice-cooled "HVJ-E suspending 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.</li> <li>● After reconstitution, the HVJ-E suspension should be stored in a refrigerator (2-8°C) for up to two (2) weeks (should be used within two weeks). For extended storage, freeze in working aliquots at -80°C for up to three (3) months. Thawing after freezing is possible only once. Repeated freezing and thawing of HVJ-E can reduce the activity.</li> </ul>
	Inadequate preparation of "Cell fusion buffer"	<ul style="list-style-type: none"> <li>● "Cell fusion buffer (20X concentrate)" should be diluted 1:20 with sterile pure water (e.g., endotoxin-free water for injection) before use.</li> </ul>
	Culture medium is too cold	<ul style="list-style-type: none"> <li>● Use of low-temperature medium for cultivating fused cells can reduce the efficiency of cell fusion. Use a pre-heated medium for the culture of fused cells.</li> </ul>
	Presence of serum in the fusion buffer	<ul style="list-style-type: none"> <li>● Contamination by &gt;2.5% (v/v) serum in the fusion buffer can reduce the efficiency of cell fusion of BHK-21 with the "Suspension Method".</li> </ul>
	Amount of HVJ-E used for fusion is not optimal  For "Suspension Method" and "Plating Method" (see Instruction Manual, p 4)	<ul style="list-style-type: none"> <li>● Adjust the volume of HVJ-E in the range of 0.5-10 µL depending on the efficiency of fusion and the degree of cytotoxicity.</li> </ul>

	<p>Cell-cell contact is weak and fusion efficiency is low</p> <p>For "Suspension Method". (see Instruction Manual, p 5)</p>	<ul style="list-style-type: none"> <li>● The efficiency of fusion may be increased if centrifugation (4°C, 2,000 rpm, 5 minutes) after Step 3 of the "Suspension Method". If centrifuged, the cells should not be re-suspended but should be incubated in the form of a pellet in Step 4. Please note that centrifuging tends to increase polynucleated cells in the case of cells with a higher potential for fusion.</li> </ul>
	<p>Splenocyte/myeloma cell mixture ratio is inadequate</p> <p>For "Protocol for preparing B cell hybridoma" (see Instruction Manual, p 6)</p>	<ul style="list-style-type: none"> <li>● If fusion efficiency (hybridoma positive rate) is low, changing the splenocyte/myeloma cell mixture ratio (10:1 to 1:1) in Step 1 of the "Protocol for preparing B cell hybridoma" .</li> </ul>
	<p>Amount of HVJ-E used for fusion is not optimal</p> <p>For "Protocol for preparing B cell hybridoma" (see Instruction Manual, p 6)</p>	<ul style="list-style-type: none"> <li>● If fusion efficiency (hybridoma positive rate) is low, adjust the volume of HVJ-E in the range of 12.5 to 50 <math>\mu</math>L in Step 5 of the "Protocol for preparing B cell hybridoma".</li> </ul>
High cytotoxicity	<p>Excessive exposure of cells to HVJ-E vector.</p> <p>For "Suspension Method". (see Instruction Manual, p 5)</p>	<ul style="list-style-type: none"> <li>● Reduce the amount of HVJ-E used. When induction of polynucleated cells or cytotoxicity is noted, remove the supernatant by centrifugation after incubation at 37°C in Step 4 of the "Suspension Method" .</li> </ul>
Induction of polynucleated cells	<p>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.</p>	<ul style="list-style-type: none"> <li>● Since HVJ-E particles strongly bind and fuse with target cell membrane within several minutes, cytotoxicity of very sensitive cell line may difficult to completely be eliminated.</li> </ul>
		<ul style="list-style-type: none"> <li>●</li> </ul>

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