

## FAQ for ***GenomONE-Neo EX***

Refer to the GenomONE web page for additional information:

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

	<b>Question</b>	<b>Answer and comment</b>
1	Characteristics of HVJ-E	GenomONE-Neo EX is a non-viral transfection reagent. Since genomic RNA of which has been completely inactivated, it has neither infective nor proliferative potentials in humans or animals.
2	Principle of transfection	The molecule to be transferred (DNA, protein, antisense oligonucleotide, siRNA, etc.) is incorporated in HVJ-E particles to yield an HVJ-E vector, which is then introduced into the target cell or tissue, making use of the sialic acid-binding activity of HN protein and membrane-fusion activity of F (fusion) protein existing in HVJ-E envelope.
3	Differences in mechanism of transfection between HVJ-E vector and other existing non-viral transfection reagents (cationic liposome etc.)	Conventional non-viral transfection reagents, including cationic lipids are incorporated into cells through endocytosis which results in degradation of most regions of the transferred DNAs or other molecules by lysosomes.  Unlike these vector systems, HVJ-E vector resists degradation by lysosomes, making it easy to transfer the specified molecules directly into the cytoplasm. Therefore, the HVJ-E vector thus yields highly efficient transfection.
4	Restriction for GenomONE-Neo EX use	GenomONE 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 for freeze-dried HVJ-E is printed on the aluminum pack of HVJ-E.
6	Particle size of HVJ-E How large is this carrier HVJ envelope vector?	The average diameter of each HVJ-E particle is approximately 300 nm (200-400 nm).
7	Method in 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 proliferate or exhibit pathogenic effects in humans or animals.  * Reference:

		<p>Prior, P. <i>et al.</i>: BioPharm, 22-33 (Oct. 1996)</p> <p>Kaneda, Y. <i>et al.</i>: Advances in Genetics, Vol. 53, pp308-332 (2005).</p>
8	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.
9	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  (2) Assay using fertilized chicken eggs  (3) Assay using mice</p>
10	Bio-safety level for laboratory use	<p>GenomONE can be used safely in ordinary laboratories.</p> <p>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 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>
11	Safety evaluation of HVJ-liposomes in nonhuman primates	<p>Safety of the HVJ-liposome vector (a hybrid vector of HVJ-E and liposomes) in cynomolgus monkeys has been demonstrated in the following paper.</p> <p>Tsuboniwa <i>et al.</i>: Human Gene Therapy, 12, 469-487 (2001).</p>
12	Quality assurance	<ul style="list-style-type: none"> <li>■ Inactivation of HVJ has been confirmed for each lot by the viral proliferative potential rule-out test, using cultured cells and fertilized chicken eggs. (Details, see the next column)</li> <li>■ Absence of contamination by bacteria and fungi has been confirmed by sterility testing.</li> <li>■ Endotoxin level has been confirmed to be less than 2.5 EU/mL (Limulus Amebocyte lysate gel clot assay).</li> <li>■ Expression of the gene introduced in cultured cells (BHK-21; ATCC CCL-10) in the presence of serum has been confirmed.</li> </ul>
13	License requirement	This product and its use are covered by the claims of one or more

	and commercial use	patents (including 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.).
14	Role of each Reagent	<ul style="list-style-type: none"> <li>■ Freeze-dried HVJ-E: The main frame of the vector into which the molecule to be transferred is included. It fuses with the cell membrane, allowing the target molecule to be introduced into the cytoplasm.</li> <li>■ Reagent A: A positively-charged peptide which increases the affinity between the target molecule and HVJ-E and thus facilitates incorporation of the molecule into HVJ-E.</li> <li>■ Reagent B: Increases permeability across the HVJ-E membrane.</li> <li>■ Reagent C: A positively-charged peptide which increases affinity between the molecule-bearing HVJ-E (HVJ-E vector) and the cell (or tissue) and thus increases the efficiency of transfection.</li> <li>■ Buffer: Neutral buffer of physiological concentration used for suspending or diluting HVJ-E or other purposes.</li> </ul>
15	Constituent and concentration of each Reagent	Not disclosed.
16	Storage of reconstituted HVJ-E suspension	<u>Do not freeze.</u> The reconstituted HVJ-E suspension <u>should be stored in a refrigerator (2-8°C) and should be used within 2 weeks.</u> Since thawing of frozen suspension can reduce activity, The suspension should not be stored frozen.
17	Storage of Reagent A, B, C and Buffer	<u>Do not freeze.</u> <u>Stored in a refrigerator (2-8°C).</u>
18	Number of HVJ-E particles included in 1 Assay Unit (AU) of HVJ-E suspension	1 Assay Unit (AU) [40µL of reconstituted HVJ-E suspension] includes approximately $10^9$ - $10^{10}$ particles of HVJ-E.
19	Hemagglutination units (HAU) of HVJ-E included in 1 Assay	1 Assay Unit (AU) [40µL of reconstituted HVJ-E suspension] is equivalent to approximately 1,000-2,000 hemagglutination unit (HAU).

	Unit (AU) of HVJ-E suspension																										
20	Efficiency of incorporation into HVJ-E	The efficiency of incorporation plasmid DNA is estimated to be approximately 15-20%. Kaneda <i>et al.</i> , Mol. Ther.: 6, 219-225 (2002)																									
21	Limit of size of DNA that can be incorporated into HVJ-E	Plasmid DNAs of 10 to 15 kbp in size are successfully incorporated into HVJ-E particles and introduced to target cells. Limit of the molecular size to be incorporated into the HVJ-E has yet to be clearly demonstrated.																									
22	Limit of size of protein or synthetic compound that can be incorporated into HVJ-E	Fluorescence-labeled proteins with their molecular weight of 7kDa (insulin) to 150kDa (rabbit IgG) are successfully incorporated into HVJ-E particles. Synthetic compounds (molecular weight >1,000) can also be incorporated into HVJ-E.																									
23	Possibility of storage of HVJ-E vector after incorporation of plasmid DNA, siRNA, ODN or protein.	HVJ-E vector (with specified molecule incorporated) should be used within the day of preparation.																									
24	Recommended cell density for each well plate size	<p><b>Adherent cells</b></p> <table border="1"> <thead> <tr> <th>Plate</th> <th>Cell density (upon inoculation onto the well plate*)</th> </tr> </thead> <tbody> <tr> <td>6-well plate</td> <td><math>0.4 - 2.0 \times 10^5</math> cells/2.0 mL of medium/well</td> </tr> <tr> <td>24-well plate</td> <td><math>1.0 \sim 5.0 \times 10^4</math> cells/0.5mL of medium/well</td> </tr> <tr> <td>96-well plate</td> <td><math>0.25 \sim 1.25 \times 10^4</math> cells/0.125mL of medium/well</td> </tr> </tbody> </table> <p>* Used for transfection under conditions of one-day culture and 50-80% confluency.</p> <p><b>Suspension cells</b></p> <p>When transfection of suspension cells is performed, the cells are combined with HVJ-E vector in a tube, and the mixture is centrifuged to induce contact between the cells and the vector, leading to transfection.</p> <table border="1"> <thead> <tr> <th rowspan="2">Plate</th> <th colspan="3">Cell density</th> </tr> <tr> <th>Centrifugation (in a tube)</th> <th>Medium for resuspension</th> <th>Inoculation onto the well plate</th> </tr> </thead> <tbody> <tr> <td>6-well</td> <td><math>0.4 \sim 2.0 \times 10^5</math> cells/0.5mL of medium/tube</td> <td>2.0mL</td> <td><math>0.4 \sim 2.0 \times 10^5</math> cells/2.0mL of medium/well</td> </tr> <tr> <td>24-well</td> <td rowspan="2"><math>0.2 \sim 1.0 \times 10^5</math> cells/0.25mL of medium/tube</td> <td rowspan="2">1.0mL</td> <td><math>1.0 \sim 5.0 \times 10^3</math> cells/0.5mL of medium/well</td> </tr> <tr> <td>96-well</td> <td><math>0.25 \sim 1.25 \times 10^5</math> cells/0.125mL of medium/well</td> </tr> </tbody> </table>	Plate	Cell density (upon inoculation onto the well plate*)	6-well plate	$0.4 - 2.0 \times 10^5$ cells/2.0 mL of medium/well	24-well plate	$1.0 \sim 5.0 \times 10^4$ cells/0.5mL of medium/well	96-well plate	$0.25 \sim 1.25 \times 10^4$ cells/0.125mL of medium/well	Plate	Cell density			Centrifugation (in a tube)	Medium for resuspension	Inoculation onto the well plate	6-well	$0.4 \sim 2.0 \times 10^5$ cells/0.5mL of medium/tube	2.0mL	$0.4 \sim 2.0 \times 10^5$ cells/2.0mL of medium/well	24-well	$0.2 \sim 1.0 \times 10^5$ cells/0.25mL of medium/tube	1.0mL	$1.0 \sim 5.0 \times 10^3$ cells/0.5mL of medium/well	96-well	$0.25 \sim 1.25 \times 10^5$ cells/0.125mL of medium/well
Plate	Cell density (upon inoculation onto the well plate*)																										
6-well plate	$0.4 - 2.0 \times 10^5$ cells/2.0 mL of medium/well																										
24-well plate	$1.0 \sim 5.0 \times 10^4$ cells/0.5mL of medium/well																										
96-well plate	$0.25 \sim 1.25 \times 10^4$ cells/0.125mL of medium/well																										
Plate	Cell density																										
	Centrifugation (in a tube)	Medium for resuspension	Inoculation onto the well plate																								
6-well	$0.4 \sim 2.0 \times 10^5$ cells/0.5mL of medium/tube	2.0mL	$0.4 \sim 2.0 \times 10^5$ cells/2.0mL of medium/well																								
24-well	$0.2 \sim 1.0 \times 10^5$ cells/0.25mL of medium/tube	1.0mL	$1.0 \sim 5.0 \times 10^3$ cells/0.5mL of medium/well																								
96-well			$0.25 \sim 1.25 \times 10^5$ cells/0.125mL of medium/well																								

		<p><b>Transfection of suspension cells</b></p>
25	Frequency of use (in vitro)	If used with the method described in this package insert, the product (GN004EX) be used for 25 assays (with a 6-well plate or a 35mm dish). If used for siRNA oligo, it can be used for 100-200 assays (with a 6-well plate).
26	Effects of serum and antibiotics in the medium during transfection (In vitro)	Usually, addition of serum and antibiotics to the transfection medium (introduction step) does not affect transfection efficiencies.
27	Efficiency of transfection of circular DNA and linear DNA (In vitro)	With regard to HVJ-E system, there are no clear evidence that transfection efficiency of linear DNA is higher than that of circular DNA.
28	Transfection efficiency of mRNAs (In vitro)	For the HVJ-E system, there is no clear evidence that transfection efficiency of mRNA is higher than that of plasmid DNA.
29	Frequency of use (in vivo/ laboratory animals)	The amount of HVJ-E required is approximately 1-2 AU (40 to 80 $\mu$ L of reconstituted HVJ-E suspension) for mice and 5-10 AU (200 to 400 $\mu$ L) for rats. Therefore, the product (GN004EX; 0.26mLx 4 vials/kit) can be used for 12-25 and 2-5 mice and rats, respectively. However, since optimal conditions of transfection in vivo can vary markedly depending on the route of administration and the type or location of the target organ, it is advisable to optimize the dose level in each experiment.
30	Precaution for in vivo transfection (route of	Because HVJ-E can be adsorbed onto tissue in vivo, especially blood cells through the HN envelope protein, the efficiency of transfection in vivo through intravenous (systemic) administration is

	administration)	<p>usually very low. It is advisable to select a route of administration involving less exposure to blood or to perform perfusion of the animal prior to administration.</p> <p>However, transfections through intravenous injection into mouse has been reported in the following articles.</p> <p>1) Matsuda N., et al.; Nuclear factor <math>\kappa</math>-B decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis. <b><i>Mol. Pharmacol.</i></b>, 67 (4), 1018-1025 (2005).</p> <p>2) Takeno M. <i>al.</i> : Th1-dominant shift of T cell cytokine production, and subsequent reduction of serum immunoglobulin E response by administration in vivo of plasmid expressing Txk/Rlk, a member of Tec family tyrosine kinase, in a mouse model. <b><i>Clin. Exp. Allergy</i></b>, 34, 965-970 (2004).</p> <p>3) Kaneda Y. et al.; Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. <b><i>Mol. Ther.</i></b>, 6 (2), 219-226 (2002).</p>
31	Immunogenicity in vivo. Consecutive administration in vivo	<p>Antibodies to HVJ-E components (<i>e.g.</i> F or HN envelope protein) will be produced after injection of the vector. However, Kaneda <i>et al.</i> have shown that luciferase gene expression is not inhibited after consecutive administration of HVJ-E into mouse muscle (twice in a 2-week interval).</p> <p>Reference; Kaneda, Y. <i>et al.</i>: <i>Advances in Genetics</i>, 53, 308-332 (2005).</p>
32	Effects of mouse or rat lineage on efficiency of transfection	<p>The type of mouse or rat lineage is not a critical factor affecting efficiency of gene expression. Other factors, including a promoter systems or purity of DNA preparation might have critical effects on this efficiency.</p>

33	Published in vivo researches using GenomONE. (Plasmid DNA)	<p>In vivo transfection using GenomONE</p> <table border="1"> <thead> <tr> <th></th> <th>Molecule</th> <th>Target tissue or organs</th> <th>Reference</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>pEBAct<sup>-</sup>insulin, with/without pEBAct<sup>-</sup>glut2</td> <td>Right lobe margin of the liver of diabetic rat</td> <td>Y. D. Kim, et al. Gene Ther. 13(3), 216-224 (2006)</td> </tr> <tr> <td>2</td> <td>pcDNA3.1/Tim44</td> <td>Carotid artery of diabetic model rat</td> <td>Matsuoka, T. et al. Diabetes, 54, 2882-2890 (2005)</td> </tr> <tr> <td>3</td> <td>HGF cDNA</td> <td>Rat left lung</td> <td>Ono, M. et al. J. Thorac. Cardiovasc. Surg. 129 (4), 740-745 (2005)</td> </tr> <tr> <td>4</td> <td>phMGFP, HGF plasmid</td> <td>Rat heart</td> <td>Futamatsu, H. et al. Circ. Res. 96, 823-830(2005).</td> </tr> <tr> <td>5</td> <td>HGF plasmid</td> <td>Rat, via the dorsalis penis superficialis vein</td> <td>Shigemura, N. et al. Am. J. Respir. Crit. Care Med.,171, 1237-1245 (2005).</td> </tr> <tr> <td>6</td> <td>Lusiferase plasmid</td> <td>Rat, injected via tail vein</td> <td>Mima, H. et al. J. Gene Med. 7(7), 888-897(2005).</td> </tr> <tr> <td>7</td> <td>pcDNA-mOPG</td> <td>Mouse sub-periosteal area</td> <td>Kanzaki,H. et al. J. Dent. Res. 83(12), 920-925 (2004).</td> </tr> <tr> <td>8</td> <td>plasmid</td> <td>Rat left lung</td> <td>Ono, M. et al. Circulation, 110, 2896-2902 (2004).</td> </tr> <tr> <td>9</td> <td>pcDNA3-tjBaM</td> <td>Mice uterine cavity</td> <td>Nakamura,H. et al. Biochem. Biophys. Res. Commun., 321(4), 886-892 (2004).</td> </tr> <tr> <td>10</td> <td>Puromycin-insensitive leucyl -specific aminopeptidase</td> <td>Mouse angiogenesis model</td> <td>Yamazaki, T. et al. Blood, 104( 8), 2345-2352 (2004).</td> </tr> <tr> <td>11</td> <td>pcDNA-mouse tk/tk</td> <td>Mouse, injection via tail vein</td> <td>Takeno, M. et al. Clin. Exp. Allergy, 34 (6), 965-970 (2004).</td> </tr> <tr> <td>12</td> <td>hVEGF, GFP DNA</td> <td>Blood mononuclear cell (ex vivo)</td> <td>Ikeda,Y. et al. Hypertens. Res., 1.27(2), 119-128 (2004)</td> </tr> <tr> <td>13</td> <td>HGF plasmid DNA</td> <td>Rat brain cisterna magna</td> <td>Shimamura, M. et al. Circulation, 109, 424-431(2004)</td> </tr> <tr> <td>14</td> <td>HGF plasmid DNA</td> <td>Rat central nervous system and the inner ear via cisterna magna</td> <td>Oshima, K. et al. FASEB J. 18(1) 212-214 (2003)</td> </tr> <tr> <td>15</td> <td>pcDNA-Luc, pcDNA-LacZ, FITC-ODN</td> <td>Mouse uterine cavity</td> <td>Nakamura, H. et al., Mol. Hum. Reprod., 9(10), 603-609 (2003)</td> </tr> <tr> <td>16</td> <td>pEGFP</td> <td>Rat embryonic cerebral cortex neurons in vitro, Rat brain thalamus</td> <td>Shimamura, M et al., Biochem. Biophys. Res. Commun, 300(2), 464-471(2003)</td> </tr> </tbody> </table>		Molecule	Target tissue or organs	Reference	1	pEBAct <sup>-</sup> insulin, with/without pEBAct <sup>-</sup> glut2	Right lobe margin of the liver of diabetic rat	Y. D. Kim, et al. Gene Ther. 13(3), 216-224 (2006)	2	pcDNA3.1/Tim44	Carotid artery of diabetic model rat	Matsuoka, T. et al. Diabetes, 54, 2882-2890 (2005)	3	HGF cDNA	Rat left lung	Ono, M. et al. J. Thorac. Cardiovasc. Surg. 129 (4), 740-745 (2005)	4	phMGFP, HGF plasmid	Rat heart	Futamatsu, H. et al. Circ. Res. 96, 823-830(2005).	5	HGF plasmid	Rat, via the dorsalis penis superficialis vein	Shigemura, N. et al. Am. J. Respir. Crit. Care Med.,171, 1237-1245 (2005).	6	Lusiferase plasmid	Rat, injected via tail vein	Mima, H. et al. J. Gene Med. 7(7), 888-897(2005).	7	pcDNA-mOPG	Mouse sub-periosteal area	Kanzaki,H. et al. J. Dent. Res. 83(12), 920-925 (2004).	8	plasmid	Rat left lung	Ono, M. et al. Circulation, 110, 2896-2902 (2004).	9	pcDNA3-tjBaM	Mice uterine cavity	Nakamura,H. et al. Biochem. Biophys. Res. Commun., 321(4), 886-892 (2004).	10	Puromycin-insensitive leucyl -specific aminopeptidase	Mouse angiogenesis model	Yamazaki, T. et al. Blood, 104( 8), 2345-2352 (2004).	11	pcDNA-mouse tk/tk	Mouse, injection via tail vein	Takeno, M. et al. Clin. Exp. Allergy, 34 (6), 965-970 (2004).	12	hVEGF, GFP DNA	Blood mononuclear cell (ex vivo)	Ikeda,Y. et al. Hypertens. Res., 1.27(2), 119-128 (2004)	13	HGF plasmid DNA	Rat brain cisterna magna	Shimamura, M. et al. Circulation, 109, 424-431(2004)	14	HGF plasmid DNA	Rat central nervous system and the inner ear via cisterna magna	Oshima, K. et al. FASEB J. 18(1) 212-214 (2003)	15	pcDNA-Luc, pcDNA-LacZ, FITC-ODN	Mouse uterine cavity	Nakamura, H. et al., Mol. Hum. Reprod., 9(10), 603-609 (2003)	16	pEGFP	Rat embryonic cerebral cortex neurons in vitro, Rat brain thalamus	Shimamura, M et al., Biochem. Biophys. Res. Commun, 300(2), 464-471(2003)
	Molecule	Target tissue or organs	Reference																																																																			
1	pEBAct <sup>-</sup> insulin, with/without pEBAct <sup>-</sup> glut2	Right lobe margin of the liver of diabetic rat	Y. D. Kim, et al. Gene Ther. 13(3), 216-224 (2006)																																																																			
2	pcDNA3.1/Tim44	Carotid artery of diabetic model rat	Matsuoka, T. et al. Diabetes, 54, 2882-2890 (2005)																																																																			
3	HGF cDNA	Rat left lung	Ono, M. et al. J. Thorac. Cardiovasc. Surg. 129 (4), 740-745 (2005)																																																																			
4	phMGFP, HGF plasmid	Rat heart	Futamatsu, H. et al. Circ. Res. 96, 823-830(2005).																																																																			
5	HGF plasmid	Rat, via the dorsalis penis superficialis vein	Shigemura, N. et al. Am. J. Respir. Crit. Care Med.,171, 1237-1245 (2005).																																																																			
6	Lusiferase plasmid	Rat, injected via tail vein	Mima, H. et al. J. Gene Med. 7(7), 888-897(2005).																																																																			
7	pcDNA-mOPG	Mouse sub-periosteal area	Kanzaki,H. et al. J. Dent. Res. 83(12), 920-925 (2004).																																																																			
8	plasmid	Rat left lung	Ono, M. et al. Circulation, 110, 2896-2902 (2004).																																																																			
9	pcDNA3-tjBaM	Mice uterine cavity	Nakamura,H. et al. Biochem. Biophys. Res. Commun., 321(4), 886-892 (2004).																																																																			
10	Puromycin-insensitive leucyl -specific aminopeptidase	Mouse angiogenesis model	Yamazaki, T. et al. Blood, 104( 8), 2345-2352 (2004).																																																																			
11	pcDNA-mouse tk/tk	Mouse, injection via tail vein	Takeno, M. et al. Clin. Exp. Allergy, 34 (6), 965-970 (2004).																																																																			
12	hVEGF, GFP DNA	Blood mononuclear cell (ex vivo)	Ikeda,Y. et al. Hypertens. Res., 1.27(2), 119-128 (2004)																																																																			
13	HGF plasmid DNA	Rat brain cisterna magna	Shimamura, M. et al. Circulation, 109, 424-431(2004)																																																																			
14	HGF plasmid DNA	Rat central nervous system and the inner ear via cisterna magna	Oshima, K. et al. FASEB J. 18(1) 212-214 (2003)																																																																			
15	pcDNA-Luc, pcDNA-LacZ, FITC-ODN	Mouse uterine cavity	Nakamura, H. et al., Mol. Hum. Reprod., 9(10), 603-609 (2003)																																																																			
16	pEGFP	Rat embryonic cerebral cortex neurons in vitro, Rat brain thalamus	Shimamura, M et al., Biochem. Biophys. Res. Commun, 300(2), 464-471(2003)																																																																			
	Published in vivo researches using GenomONE. (siRNA)	<p>Ito, M. <i>et al.</i>: Rad51 siRNA delivered by HVJ envelope vector enhances the anti-cancer effects of cisplatin. <i>J. Gene Medicine</i>, 7 (7), 889-897 (2005). [HeLa S3/scid mouse, intra-tumor injection of siRNA]]</p>																																																																				
	Published researches using GenomONE	<p>Various applications are shown in GenomONE web page. <a href="http://www.iskweb.co.jp/hvj-e/genomonereferences.htm">http://www.iskweb.co.jp/hvj-e/genomonereferences.htm</a></p>																																																																				



**ISHIHARA SANGYO KAISHA, LTD.**

URL: <http://www.iskweb.co.jp/hvj-e/>

E-MAIL: [HVJ-E@iskweb.co.jp](mailto:HVJ-E@iskweb.co.jp)