Transfection of siRNA/miRNA into immune cells

**GenomONE - Si**

Data sheet

For research use

URL: https://www.iskweb.co.jp/eng/products/hvj-e/
E-mail: HVJ-E@iskweb.co.jp
HVJ Envelope siRNA/miRNA transfection kit

GenomONE-Si

<Features>
- Optimal for transfection of synthetic oligo-type siRNA/miRNA
- Simple operability (completion of transfection within 5 to 10 minutes)
- Applicable also to suspended immune cells, transfection into which is usually difficult
- Optimal for rapid screening of a large number of test samples (HTS: High-throughput screening)
- Low cytotoxicity and high safety

GenomONE-Si is a kit for dedicated use for transfection of siRNA/miRNA using the HVJ envelope (HVJ-E: inactivated hemagglutinating virus of Japan), with which highly efficient transfection is possible even into primary cultured immune cells.

Standard Protocol

Steps (1) through (4) should be performed on ice.
- The basic protocol is completed through 5 steps (incubation is unnecessary).
- A protocol for high-throughput screening (HTS) is also available.

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>HVJ-E (inactivated HVJ)</th>
<th>Reagent D (reagent for incorporation)</th>
<th>Reagent E (enhancer for introduction)</th>
<th>Buffer (for suspension and dilution)</th>
<th>Number of times of use for assay (wells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freeze-dried 0.26 mL/vial (when reconstituted)</td>
<td>0.5 mL/vial</td>
<td>4.0 mL/vial</td>
<td>6.5 mL/vial</td>
<td>6-well plate</td>
</tr>
<tr>
<td>GS001</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>100</td>
</tr>
<tr>
<td>GS004</td>
<td>4 vials</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>400</td>
</tr>
<tr>
<td>GS016</td>
<td>16 vials</td>
<td>4 vials</td>
<td>4 vials</td>
<td>4 vials</td>
<td>1,600</td>
</tr>
<tr>
<td>GS040</td>
<td>40 vials</td>
<td>10 vials</td>
<td>10 vials</td>
<td>10 vials</td>
<td>4,000</td>
</tr>
</tbody>
</table>
**What is HVJ-E (inactivated Sendai virus)**

Hemagglutinating virus of Japan (HVJ) Envelope (HVJ-E) is a non-proliferative and non-infectious vesicle about 300 nm in diameter on average purified after complete inactivation of Sendai virus genomic RNA. Since the F protein distributed on the HVJ-E envelope has high membrane-fusing potential comparable to that of live virus, it is possible to use HVJ-E itself as a cell-fusing agent or to introduce genes, proteins, anti-cancer agents, etc. in HVJ-E-incorporated form into cells for analysis of their functions.

**Introduction into cells making use of the membrane-fusing potential of HVJ-E**

1. Incorporation of the target molecule
2. Adsorption onto cells
3. Introduction into cells through membrane fusion

**References (Review articles)**


Transfection of siRNA into immune cell strains (U937, Raji, THP-1, Jurkat, and HL-60)
**U937 siRNA transfection**

**Transfection of Eg5 siRNA into U937 Cells**

Eg5 siRNA (50nM) transfection → 2days → WST-8 (viable cell count, A<sub>450</sub>)

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

**Cell**: U-937 (Human leukemic monocytic lymphoma cell line, ATCC: CRL-1593.2)

**Culture condition**: 4 × 10<sup>3</sup> cells/well/100μL, 10%FBS-RPMI-1640

**Culture plate**: 96-well plate (IWAKI 3860-096)

**siRNA**: Silencer KIF11 (Eg5) siRNA (Ambion Code No. AM4639)

Negative control #1 siRNA (Ambion Code No. AM4639)

A knockdown efficiency of 94% was obtained by the transfection of Eg5 siRNA into U937 cells using GenomONE-Si, whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

As a kinesin-like motor protein, Eg5 (KIF11) is essential for the formation of spindle microtubules during cell division, and when its function is inhibited, cell division is stopped and apoptosis is induced. Based on such a phenomenon, the efficiency of transfection of siRNA is quantitatively evaluated by the WST-8 method (a method for determination of viable cell count).
Raji
siRNA transfection

**Transfection of CDC2 siRNA into Raji Cells**

CDC2 siRNA (50nM) transfection → 2days → RT-PCR (Quantitation of mRNA)

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

**Cell**: Raji (Human Burkitt lymphoma cell line, ATCC:CCL-86)

**Culture condition**: 1.25 × 10^5 cells/well/500μL, 10%FBS-RPMI-1640

**Culture plate**: 24-well plate (FALCON 3047)

**siRNA**: Very High Potency Hs_CDC2 siRNA (QIAGEN Cat. No. 1027273)

Negative control #1 siRNA (Ambion Code No. AM4639)

No.GS-S-002

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<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (10μM)</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
</tr>
</tbody>
</table>

Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

Steps (1) through (4) should be performed on ice.

A knockdown efficiency of 75% was obtained by the transfection of CDC2 siRNA into Raji cells using GenomONE-Si, whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

CDC2 forms a complex with cyclin B, through which the M phase of cell cycle is suppressed, and such a knockdown efficiency is quantitatively evaluated by the real-time PCR method.
THP-1
siRNA transfection

Transfection of Eg5 siRNA into THP-1 Cells

Eg5 siRNA (50 nM) transfection → 2 days → WST-8 (viable cell count, $A_{450}$)

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

[Cell]: THP-1 (Human acute monocytic leukemia cell line, ATCC: TIB-202)
[Culture condition]: $2.5 \times 10^4$ cells/well/100 μL, 10% FBS-RPMI-1640
[Culture plate]: 96-well plate (IWAKI 3860-096)
[siRNA]: Silencer KIF11 (Eg5) siRNA (Ambion Code No. AM4639)
Negative control #1 siRNA (Ambion Code No. AM4639)

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (96-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (10 μM)</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5% CO2</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

A knockdown efficiency of 94% was obtained by the transfection of Eg5 siRNA into THP-1 cells using GenomONE-Si, whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

As a kinesin-like motor protein, Eg5 (KIF 11) is essential for the formation of spindle microtubules during cell division, and when its function is inhibited, cell division is stopped and apoptosis is induced. Based on such a phenomenon, the efficiency of transfection of siRNA is quantitatively evaluated by the WST-8 method (a method for determination of viable cell count).
**Transfection of Eg5 siRNA into Jurkat Cells**

Eg5 siRNA (50nM) transfection → 2 days → WST-8 (viable cell count, $A_{450}$)

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

**Steps**

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (96-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
</tr>
<tr>
<td></td>
<td>HVJ-E: 2.5μL</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td></td>
<td>Reagent D: 0.5μL</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (10μM)</td>
</tr>
<tr>
<td></td>
<td>siRNA solution: 10μL</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td></td>
<td>Reagent E: 5μL</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5% CO2</td>
</tr>
<tr>
<td></td>
<td>Suspension[(1)+(2)+(3)+(4)]: 0.9μL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

A knockdown efficiency of 73% was obtained by the transfection of Eg5 siRNA into Jurkat cells using GenomONE-Si, whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

As a kinesin-like motor protein, Eg5 (KIF 11) is essential for the formation of spindle microtubules during cell division, and when its function is inhibited, cell division is stopped and apoptosis is induced. Based on such a phenomenon, the efficiency of transfection of siRNA is quantitatively evaluated by the WST-8 method (a method for determination of viable cell count).
HL-60
siRNA transfection

Transfection of CDC2 siRNA into HL-60 Cells
CDC2 siRNA(50nM) transfection → 2days → RT-PCR(Quantitation of mRNA)

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

[Cell]: HL-60 (Human promyelocytic leukemia cell line, ATCC:CCL-240)
[Culture condition]: $1 \times 10^5$cells/well/500μL, 20%FBS-RPMI-1640
[Culture plate]: 24-well plate(FALCON 3047)
[siRNA]: Very High Potency Hs_CDC2 siRNA (QIAGEN Cat. No. 1027273)
Negative control #1 siRNA(Ambion Code No.AM4639)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
<td>HVJ-E : 2.5μL</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
<td>Reagent D: 0.5μL</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (10μM)</td>
<td>siRNA solution: 10μL</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
<td>Reagent E: 5μL</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
<td>Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

A knockdown efficiency of 85% was obtained by the transfection of CDC2 siRNA into HL-60 cells using GenomONE-Si, whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

CDC2 forms a complex with cyclin B, through which the M phase of cell cycle is suppressed, and such a knockdown efficiency is quantitatively evaluated by the real-time PCR method.

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No.GS-S-005
Transfection of siRNA / miRNA into Mouse Primary B and T Cells
Methods

1. CD4⁺ T cells were isolated from the lymph nodes of Foxp3-GFP reporter mice.
2. Isolated CD4⁺ T cells were transfected with CDK8/19 siRNAs using GenomONE-Si (250nM).
3. siRNA-transfected T cells were stimulated with Dynabeads® Mouse T-Activator CD3/CD28 in the presence of IL-2 (50U/mL) and TGF-β (2ng/mL) [2 × 10⁴ cells/well; 96 well plate].
4. After a 24 hr incubation, cells were transfected with CDK8/19 siRNA using GenomONE-Si (second transfection).
5. Forty-eight hours after transfection, Foxp3 mRNA expression was measured by real-time PCR, and Foxp3⁺ cells were detected by flow cytometry.

Isolated CD4⁺ T cells → siRNA(250nM) transfection & stimulation 24 hr → siRNA(250nM) transfection 48 hr → Real-time PCR analysis of Foxp3 mRNA expression → Flow cytometry analysis of Foxp3⁺ cells

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

Cell: CD4⁺ T cells from the lymph nodes of the Foxp3-GFP reporter mouse.
Culture condition: 2 × 10⁴ cells/well/100μL, RPMI-1640, 10% FBS, penicillin G (60μg/mL), streptomycin (100μg/mL), 0.1mM 2-ME
Stimulation: IL-2 (50 U/mL), TGF-β (2 ng/mL), Dynabeads® Mouse T-Activator CD3/CD28 (Thermo Fisher Scientific)
Culture plate: 96-well plate
siRNA: CDK8 siRNA (Thermo Fisher Scientific. ID No. s113914)
CDK19 siRNA (Thermo Fisher Scientific, ID No. s95476)
Negative control siRNA (Thermo Fisher Scientific)
Transfection: GenomONE-Si (Ishihara sangyo)

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
<th>Amount of reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
<td>HVJ-E: 2.5μL</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
<td>Reagent D: 0.5μL</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (50μM)</td>
<td>siRNA solution: 10μL</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
<td>Reagent E: 5μL</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated at 37°C under 5%CO₂</td>
<td>Suspension[(1)+(2)+(3)+(4)]: 2μL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

To perform a double knockdown of CDK8 and CDK19: 2μL of each HVJ-E suspension containing the CDK8- or CDK19-targeting siRNA were transfected.

ISK

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**Results**

Fig. 1 Knockdown of CDK8 or 19 expression in mouse primary T cells.

Transfection of CDK8/19-targeting siRNA using GenomONE-Si suppressed CDK8/19 expression in mouse primary T cells (Fig. 1). Knockdown of CDK8/19 expression facilitated Treg conversion (Fig. 2).

**Conclusion**

GenomONE-Si enabled efficient siRNA transfection even in difficult-to-transfect cells such as mouse primary T cells.
Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.

The mimic housekeeping positive control #1 (PPIB) miRNA was transfected using GenomONE-Si into unstimulated mouse primary B cells, giving a knockdown efficiency of 66%, whereas the knockdown efficiency of transfection of the cyclophilin B control siRNA was 21%, which is inadequate.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.
# BALB/c mouse primary B cells

**miRNA transfection**

### Transfection of Cyclophilin B miRNA into pre-stimulated mouse primary B cells

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HVJ-E suspension taken into a micro-test tube</td>
<td>HVJ-E: 2.5µL</td>
</tr>
<tr>
<td>2. Combination with Reagent D and agitation (tapping)</td>
<td>Reagent D: 0.5µL</td>
</tr>
<tr>
<td>3. Combination with miRNA solution and agitation (tapping) (2µM)</td>
<td>miRNA solution: 10µL</td>
</tr>
<tr>
<td>4. Combination with Reagent E and agitation (tapping)</td>
<td>Reagent E: 5µL</td>
</tr>
<tr>
<td>5. HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
<td>Suspension[(1)+(2)+(3)+(4)]: 4.5µL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

The mimic housekeeping positive control #1 (PPIB) miRNA was transfected using GenomONE-Si into mouse primary B cells that had been stimulated with [1] anti-CD40/LPS or [2] anti-CD40/IL4, consistently giving a knockdown efficiency of not less than 70%.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.
**Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary B cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for **GenomONE-Si**.

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with miRNA solution and agitation</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with cell</td>
</tr>
<tr>
<td></td>
<td>culture in a well and incubated for 48 hours</td>
</tr>
<tr>
<td></td>
<td>at 37°C under 5%CO2</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

The mimic housekeeping positive control #1 (PPIB) miRNA was transfected using **GenomONE-Si** into mouse primary B cells that had been stimulated with LPS, giving a knockdown efficiency of 82%, whereas no adequate efficiency was obtained using other transfection reagents; thus, the superiority of **GenomONE-Si** was demonstrated. These procedures were carried out in the presence of LPS before and after transfection.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.
## BALB/c mouse primary B cells

### miRNA transfection

**Cyclophilin B**

- a: Control (PBS)
- b: Mock
- c: Negative control miRNA (50nM)
- d: Cyclophilin B miRNA (50nM)
- e: Cyclophilin B miRNA (10nM)

**β-actin**

### Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary B cells

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### miRNA transfection procedure

**Steps**

1. HVJ-E suspension taken into a micro-test tube
2. Combination with Reagent D and agitation (tapping)
3. Combination with miRNA solution and agitation (tapping) (10-2μM)
4. Combination with Reagent E and agitation (tapping)
5. HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2

**Amount of reagent (24-well plate)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension</td>
<td>2.5μL</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D</td>
<td>0.5μL</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with miRNA solution</td>
<td>10μL</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E</td>
<td>5μL</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension</td>
<td>4.5μL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

The mimic housekeeping positive control #1 (PPIB) miRNA (10, and 50 nM) was transfected using GenomONE-Si into mouse primary B cells that had been stimulated with LPS, and the knockdown efficiency was confirmed by the Western blotting method. These procedures were carried out in the presence of LPS before and after transfection.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was evaluated by the Western blotting method.

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BALB/c mouse primary B cells
miRNA transfection

Transfection of Cyclophilin B miRNA into Post-stimulation Mouse primary B cells

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

[Cell]: Mouse primary B cells [isolated from spleen of female BALB/c mouse (12 weeks old)]

[Stimulation]: LPS (1µg/mL)

[Culture condition]: 1 × 10^6 cells/well/500µL, RPMI-1640 with 10% FBS, 10mM HEPES, 50µM 2-ME

[Culture plate]: 24-well plate

[miRNA]: Mimic Housekeeping Positive Control #1 (PPIB) (Thermo Scientific Cat No.CP-002000-01-05)

miRIDIAN microRNA Mimic Negative Control #1 (Thermo Scientific Cat No.CN-001000-01-05)

The mimic housekeeping positive control #1 (PPIB) miRNA was transfected using GenomONE-Si into unstimulated mouse primary B cells, and the knockdown efficiency was confirmed by the Western blotting method after stimulation with LPS for 1 to 3 days.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the Western blotting method.

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No.GS-M-004
**BALB/c mouse primary T cells**

**siRNA/miRNA transfection**

**Transfection of Cyclophilin B siRNA/miRNA into Pre-stimulated mouse primary T cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for **GenomONE-Si**.

**[Cell]:** Mouse primary T cells [isolated from spleen of female BALB/c mouse (7 weeks old)]

**[Stimulation]:** PMA (5nM) / ionomycin (1µg/mL)

**[Culture condition]:** 1 × 10^6 cells/well/500 µL, RPMI-1640 with 10% FBS, 1% GlutaMAX™ (100 x)

**[Culture plate]:** 24-well plate

**[siRNA]:** siGENOME Cyclophilin B control siRNA (Thermo Scientific Cat No.D-001136-01-05)

Negative control #1 siRNA (Ambion Code No.AM4639)

**[miRNA]:** Mimic Housekeeping Positive Control #1 (PPIB) (Thermo Scientific Cat No.CP-002000-01-05)

miRIDIAN microRNA Mimic Negative Control #1 (Thermo Scientific Cat No.CN-001000-01-05)

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**Table:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube: HVJ-E: 2.5µL</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping): Reagent D: 0.5µL</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA/miRNA solution and agitation (tapping) (30µM): siRNA/miRNA solution: 10µL</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping): Reagent E: 5µL</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5% CO2: Suspension[(1)+(2)+(3)+(4)]: 4.5µL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the cyclophilin B control siRNA was transfected using **GenomONE-Si** into the T cells, giving a knockdown efficiency of 77%. Furthermore, the mimic housekeeping positive control #1 (PPIB) miRNA was transfected, giving a knockdown efficiency of 96%.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.

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No.GS-S/M-002
**BALB/c mouse primary T cells**

**siRNA transfection**

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**Transfection of Cyclophilin B siRNA into Pre-stimulated mouse primary T cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

- **Cell**: Mouse primary T cells [isolated from spleen of female BALB/c mouse (12 weeks old)]
- **Stimulation**: PMA(5nM) / ionomycin(1µg/mL)
- **Culture condition**: 8×10^5 cells/well/500µL, RPMI-1640 with 10% FBS, 1% GlutaMAX™(100×)
- **Culture plate**: 24-well plate
- **siRNA**: siGENOME Cyclophilin B control siRNA (Thermo Scientific Cat No.D-001136-01-05)
  Negative control #1 siRNA(Ambion Code No.AM4639)

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<table>
<thead>
<tr>
<th>Step</th>
<th>Amount of reagent (24-well plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
</tr>
<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with siRNA solution and agitation (tapping) (30~1.1µM)</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the cyclophilin B control siRNA was transfected using GenomONE-Si into the T cells, giving a knockdown efficiency of not less than 70% by the RT-PCR method. When 50 nM of siRNA was transfected, a maximum knockdown efficiency of 86% was obtained.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.

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No.GS-S-006
C57BL/6 mouse primary T cells
miRNA transfection

Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary T cells

Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the mimic housekeeping positive control #1 (PPIB) miRNA was transfected using GenomONE-Si into the T cells, giving a knockdown efficiency of not less than 70% by the RT-PCR method. When 10 nM of miRNA was transfected, a maximum knockdown efficiency of 94% was obtained.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.

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**BALB/c mouse primary T cells**

**miRNA transfection**

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<td>(1)</td>
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<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with miRNA solution and agitation (tapping) (200~0.3nM)</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the mimic housekeeping positive control #1 (PPIB) miRNA(400 pM) was transfected using GenomONE-Si into the T cells, giving a knockdown efficiency of 75% by the RT-PCR method. Furthermore, the knockdown was also confirmed by the Western blotting method.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method and was also evaluated further by the Western blotting method.

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**Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary T cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

- **Cell**: Mouse primary T cells [isolated from spleen of female BALB/c mouse (6 weeks old)]
- **Stimulation**: PMA(5nM) / ionomycin(1µg/mL)
- **Culture condition**: 2 × 10^6 cells/well/500µL, RPMI-1640 with 10% FBS, 1% GlutaMAX™(100×)
- **Culture plate**: 24-well plate
- **miRNA**: Mimic Housekeeping Positive Control #1 (PPIB) (Thermo Scientific Cat No.CP-002000-01-05) miRIDIAN microRNA Mimic Negative Control #1 (Thermo Scientific Cat No.CN-001000-01-05)

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No.GS-M-006
**Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary T cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for **GenomONE-Si**.

**Steps**

<table>
<thead>
<tr>
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<tr>
<td>(1)</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
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<tr>
<td>(2)</td>
<td>Combination with Reagent D and agitation (tapping)</td>
</tr>
<tr>
<td>(3)</td>
<td>Combination with miRNA solution and agitation (tapping) (2μM)</td>
</tr>
<tr>
<td>(4)</td>
<td>Combination with Reagent E and agitation (tapping)</td>
</tr>
<tr>
<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the mimic housekeeping positive control #1 (PPIB) miRNA was transfected using **GenomONE-Si** into the T cells, giving a knockdown efficiency of 79% by the RT-PCR method. Whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of **GenomONE-Si** was demonstrated.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was quantitatively evaluated by the real-time PCR method.

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No.GS-M-007
**BALB/c mouse primary T cells**

miRNA transfection

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**Cyclophilin B**

- a, b, c, d, e: GenomONE-Si

**β-actin**

- f, g, h, i, j: GenomONE-Si

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**Transfection of Cyclophilin B miRNA into Pre-stimulated mouse primary T cells**

The transfection procedures were carried out according to Protocol (1) in the Instruction Manual for GenomONE-Si.

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<td>Combination with Reagent E and agitation (tapping)</td>
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<td>(5)</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
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Steps (1) through (4) should be performed on ice.

After stimulation of mouse spleen cells with PMA/ionomycin for 1 day, T cells were separated, then the mimic housekeeping positive control #1 (PPIB) miRNA(2, and 50 nM) was transfected using GenomONE-Si into the T-cells, and the knockdown efficiency was confirmed by the Western blotting method.

Whereas no adequate efficiency could be obtained using other transfection reagents; thus, the superiority of GenomONE-Si was demonstrated.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was evaluated by the Western blotting method.

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**BALB/c mouse primary T cells**

**miRNA transfection**

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<tbody>
<tr>
<td>1</td>
<td>HVJ-E suspension taken into a micro-test tube</td>
<td>HVJ-E (× 1): 2.5μL, HVJ-E (× 1/2 vol.): 1.25μL</td>
</tr>
<tr>
<td>2</td>
<td>Combination with Reagent D and agitation (tapping)</td>
<td>Reagent D: 0.5μL, Reagent D: 0.25μL</td>
</tr>
<tr>
<td>2'</td>
<td>Combination with Buffer and agitation (tapping)</td>
<td>Buffer: 1.5μL</td>
</tr>
<tr>
<td>3</td>
<td>Combination with miRNA solution and agitation (tapping) (10μM)</td>
<td>miRNA solution: 10μL</td>
</tr>
<tr>
<td>4</td>
<td>Combination with Reagent E and agitation (tapping)</td>
<td>Reagent E: 5μL, 2-fold dilutions of Reagent E: 5μL</td>
</tr>
<tr>
<td>5</td>
<td>HVJ-E vector suspension is combined with the cell culture in a well and incubated for 48 hours at 37°C under 5%CO2</td>
<td>Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well</td>
</tr>
</tbody>
</table>

Steps (1) through (4) should be performed on ice.

The mimic housekeeping positive control #1 (PPIB) miRNA was transfected using **GenomONE-Si** into unstimulated mouse primary T cells, and after stimulation with PMA/ionomycin for 24 to 48 hours, the knockdown efficiency was confirmed by the Western blotting method.

Cyclophilin B (PPIB: peptidylprolyl isomerase B) is one type of housekeeping gene, the knockdown efficiency of which was evaluated by the Western blotting method.