For research use

HVJ Envelope siRNA/miRNA transfection kit

GenomONE - Si

Transfection of siRNA/miRNA into immune cells

Data sheet

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200218GS-P-M

HVJ Envelope siRNA/miRNA transfection kit

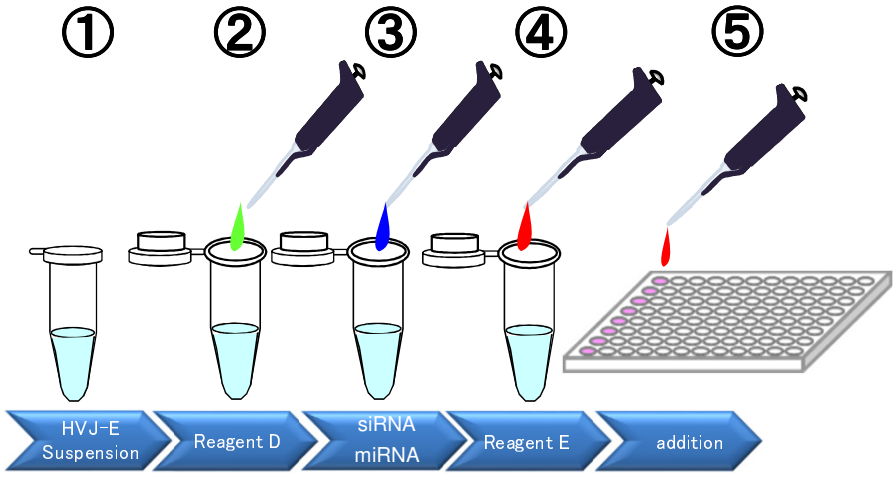
GenomONE - Si

<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



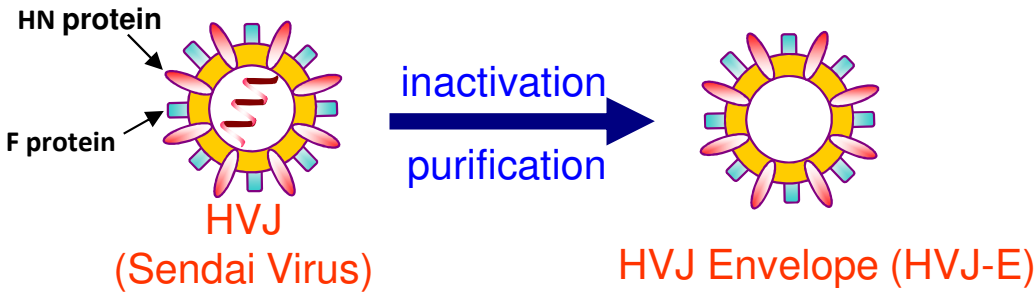
Steps (1) through (4) should be performed on ice.

- The basic protocol is completed through 5 steps (incubation is unnecessary).
- A protocol for high-throughput screening (HTS) is also available.

Cat. #	HVJ-E (inactivated HVJ) Freeze-dried 0.26 mL /vial (when reconstituted)	Reagent D (reagent for incorporation) 0.5 mL/vial	Reagent E (enhancer for introduction) 4.0 mL/vial	Buffer (for suspension and dilution) 6.5 mL/vial	Number of times of use for assay (wells)		
					6-well plate	24-well plate	96-well plate
GS001	1 vial	1 vial	1 vial	1 vial	100	400	2,000
GS004	4 vials	1 vial	1 vial	1 vial	400	1,600	8,000
GS016	16 vials	4 vials	4 vials	4 vials	1,600	6,400	32,000
GS040	40 vials	10 vials	10 vials	10 vials	4,000	16,000	80,000

GenomONE-Si (HVJ-E siRNA/miRNA transfection kit)

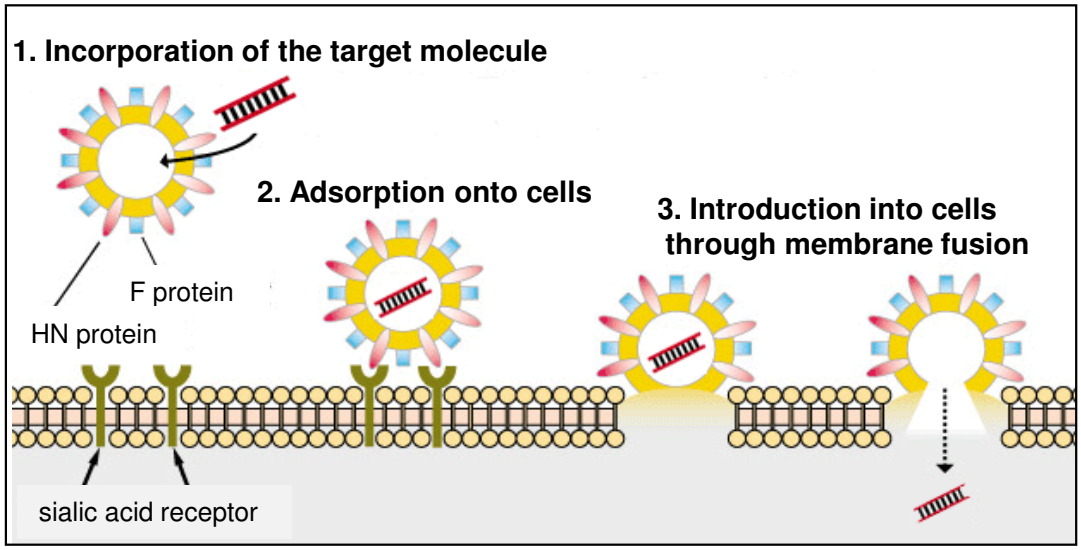
What is HVJ-E (inactivated Sendai virus)



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.

HVJ(Hemagglutinating virus of Japan)is also called Sendai virus(SeV)or Mouse Parainfluenza virus type 1.

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



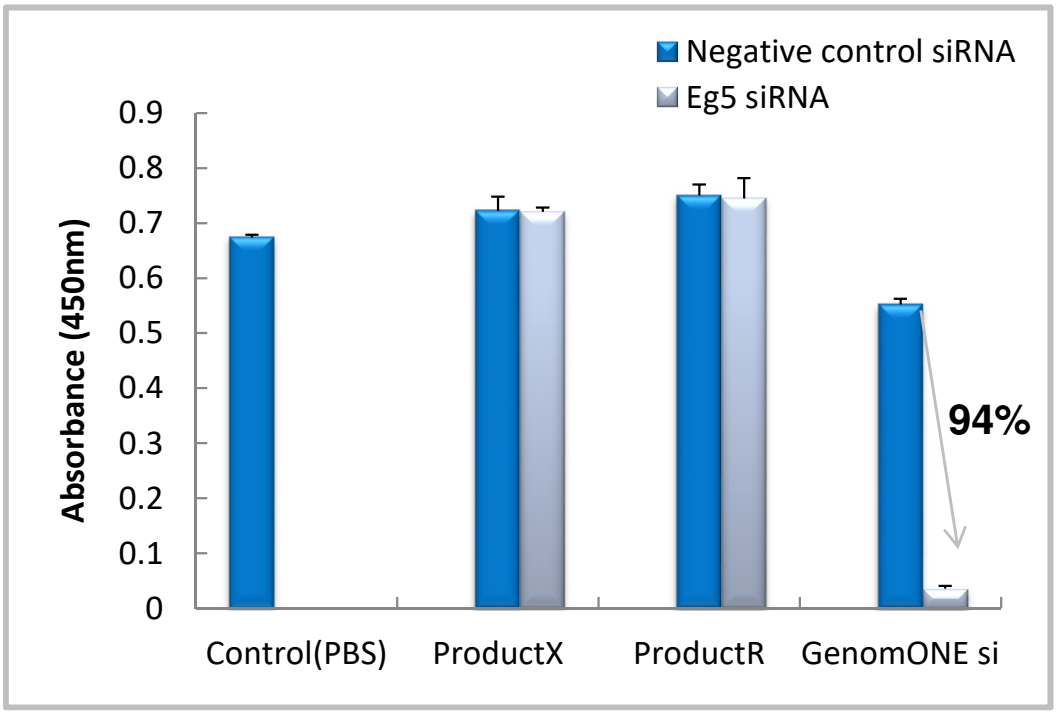
References (Review articles)

Kaneda Y. *et al.* : Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. ***Molecular Therapy***, 6, 219-226 (2002).
Kaneda Y. : New vector innovation for drug delivery: development of fusigenic non-viral particles. ***Curr. Drug Targets***, 4(8), 599-602 (2003).
Kaneda Y. : Applications of Hemagglutinating Virus of Japan in therapeutic delivery systems. ***Expert Opin. Drug Deliv.***, 5(2),221-233 (2008).
Zhang Q. *et al.* : HVJ envelope vector, a versatile delivery system: its development, application and perspectives. ***Biochem. Biophys. Res. Commun.***, 373, 345-349 (2008).
Kato F. *et al.* : Hemagglutinating Virus of Japan Envelope Vectors as High-Performance Vehicles for Delivery of Small RNAs. ***J. Genet. Syndr. Gene Ther.***, 4,178 (2013).

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_{450}）

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】: 4×10^3cells/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)

	Step	Amount of reagent (96-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (10μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 0.9μL/well

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

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 (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).

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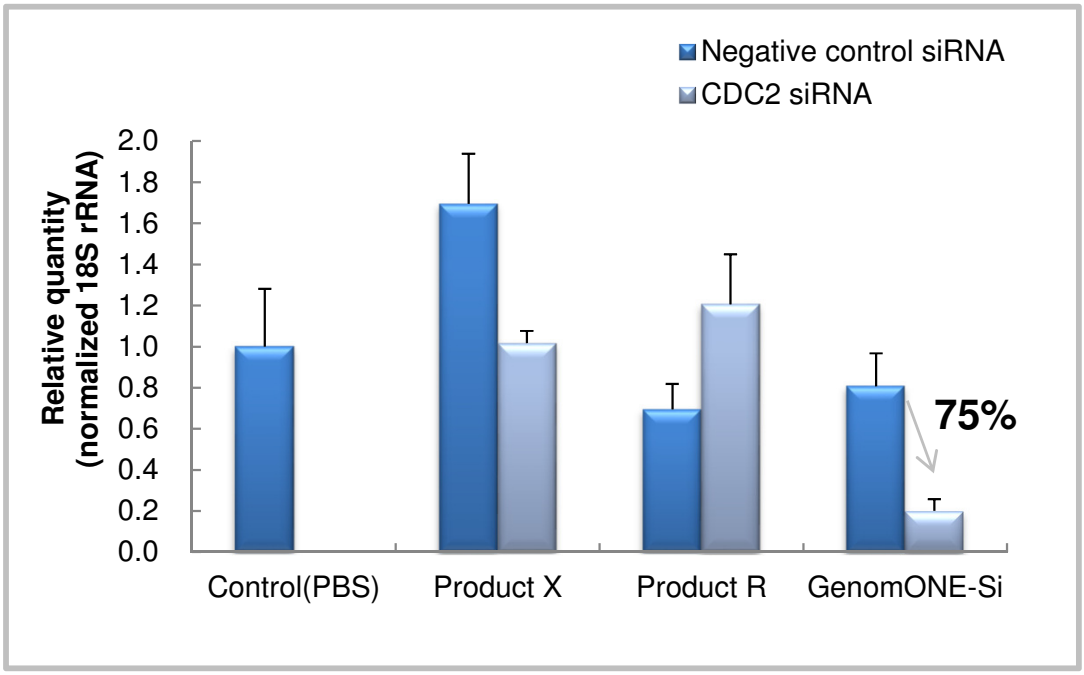
Raji

siRNA transfection



Relative Quantity; qRT-PCR assays (n=3)

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 × 10^5cells/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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (10μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	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.

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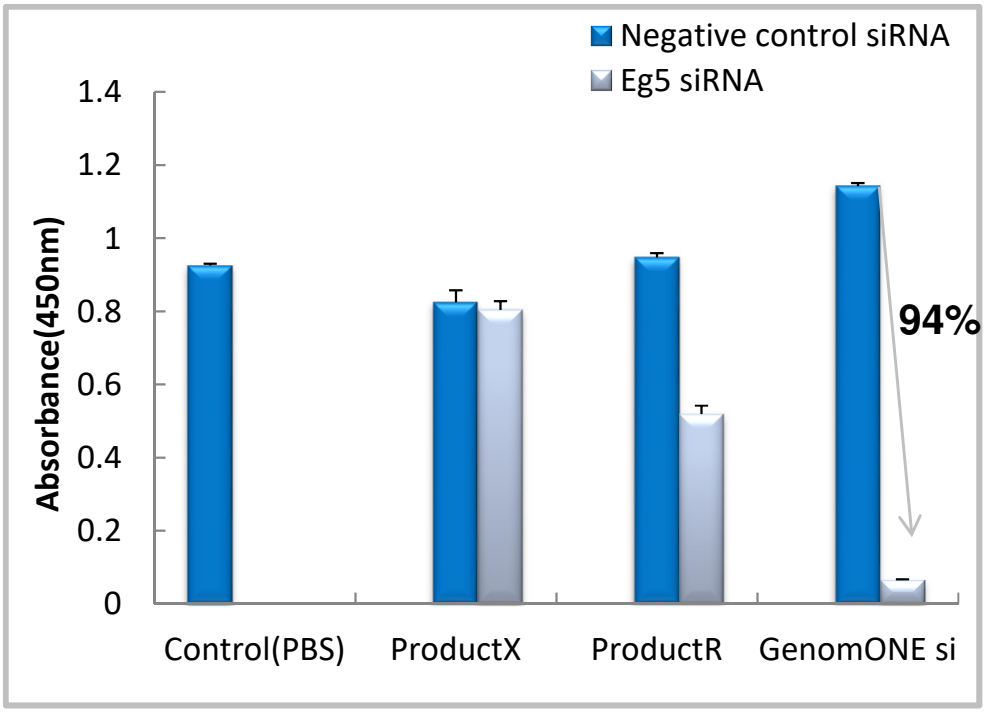
THP-1

siRNA transfection



Transfection of Eg5 siRNA into THP-1 Cells

Eg5 siRNA(50nM) transfection → 2days → 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】: 2.5×10^4cells/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)

	Step	Amount of reagent (96-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (10μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 0.9μL/well

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).

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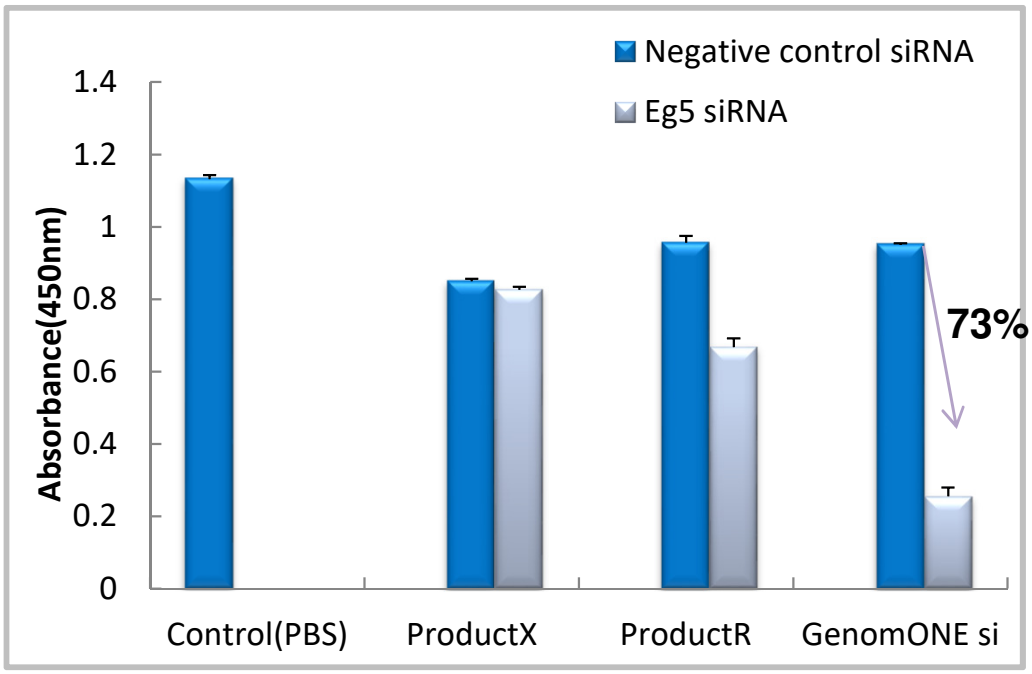
Jurkat

siRNA transfection



Transfection of Eg5 siRNA into Jurkat Cells

Eg5 siRNA(50nM) transfection → 2days → 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】: Jurkat clone E6-1(Human T cell leukemia cell line, ATCC:TIB-152)
【Culture condition】: 2×10^4cells/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)

	Step	Amount of reagent (96-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (10μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 0.9μL/well

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).

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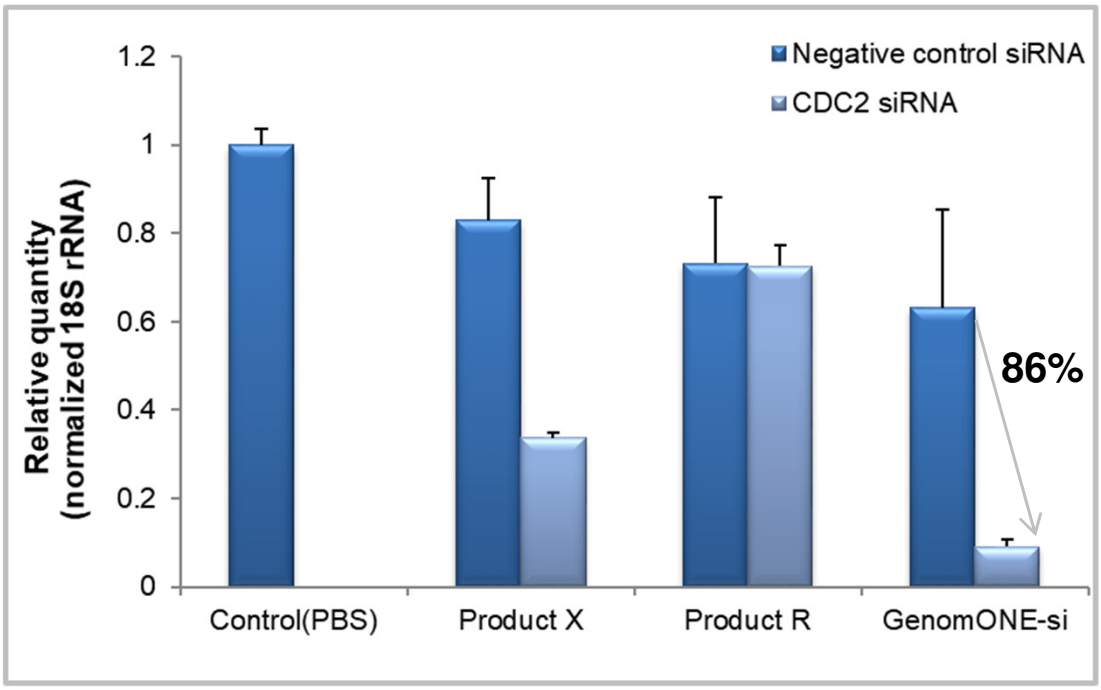
HL-60

siRNA transfection



Relative Quantity; qRT-PCR assays (n=3)

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】: 1×10^5cells/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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (10μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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Transfection of siRNA / miRNA into Mouse Primary B and T Cells

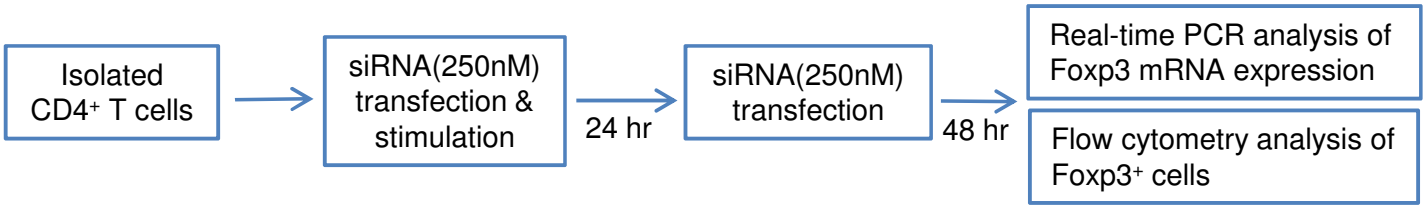
siRNA transfection into mouse primary T cells

Foxp3 induction by knockdown of CDK8/19 expression

Data: Dr. Norihisa Mikami
Department of Experimental Immunology, WPI Immunology Frontier Research Center, Osaka University
Related article: *Sci. Immunol*, 4, eaaw2707 (2019).

【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) [2×10^4 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.



CDK: Cyclin-dependent kinase
Foxp3: transcription factor forkhead box protein 3

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】: 2×10^4cells/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)

Step	Procedure	Amount of reagent
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5µL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5µL
(3)	Combination with siRNA solution and agitation (tapping) (50µM)	siRNA solution: 10µL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5µL
(5)	HVJ-E vector suspension is combined with the cell culture in a well and incubated at 37°C under 5%CO_2	Suspension[(1)+(2)+(3)+(4)]: 2µL/well

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.

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【Results】

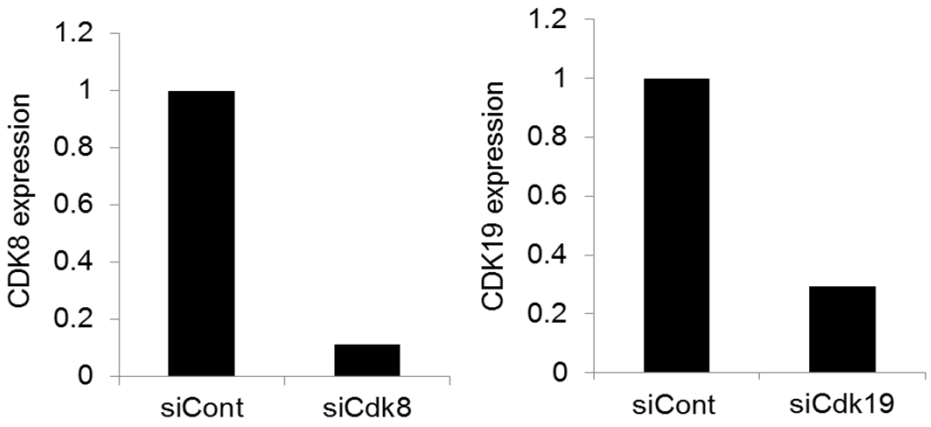

Fig.1 Knockdown of CDK8 or 19 expression in mouse primary T cells.

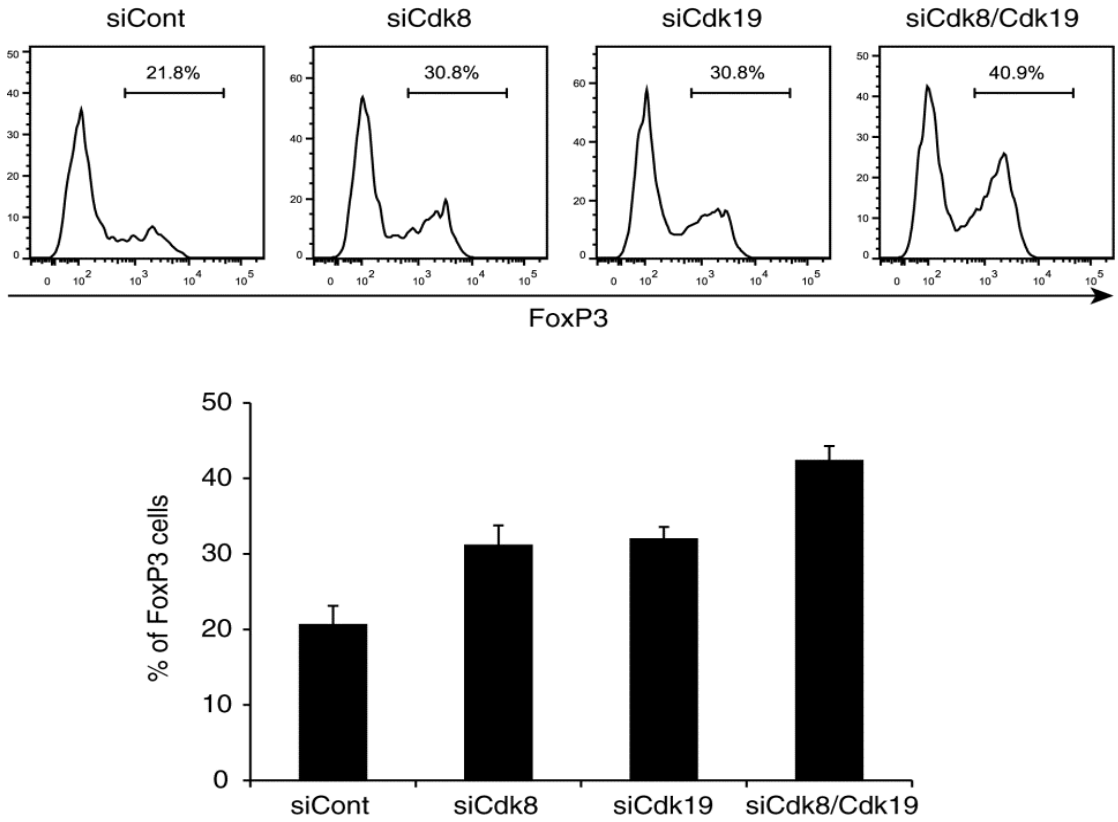

Fig.2 Effects of CDK8/19 knockdown on Foxp3 expression in mouse primary T cells.

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.

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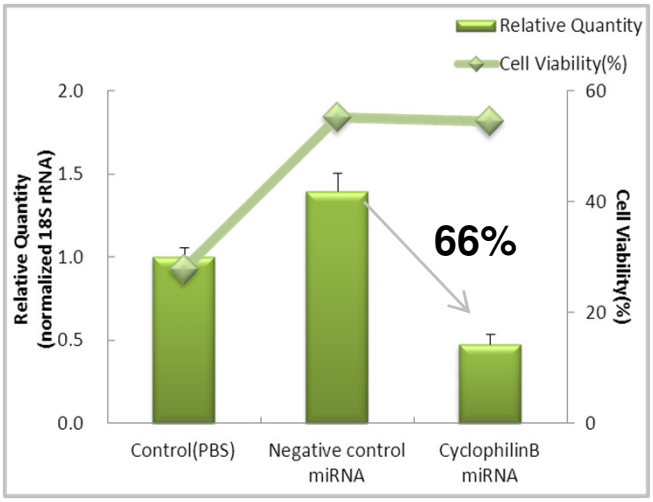
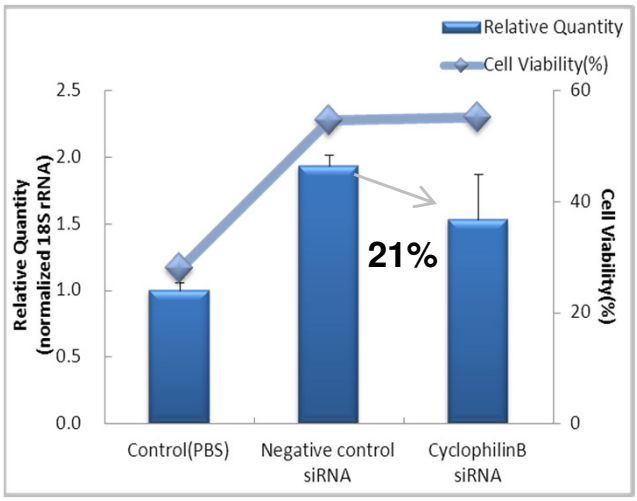
BALB/c mouse primary B cells

siRNA/miRNA transfection

miRNA transfection



siRNA transfection



Relative Quantity; qRT-PCR assays (n=3)
Cell Viability (%); PI staining assay

Transfection of Cyclophilin B siRNA/miRNA into Mouse Primary B Cells

B cells isolation & Transfection → **No-stimulation** **2 days** → RT-PCR (Quantitation of mRNA)

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 (6 weeks old)]
【Culture condition】: 5×10^6 cells/well/500µL, RPMI-1640 with 10% FBS, 10mM HEPES, 50µM 2-ME
【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)

	Step	Amount of reagent （24-well plate）
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5µL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5µL
(3)	Combination with siRNA/miRNA solution and agitation (tapping) (10µM)	siRNA/miRNA solution: 10µL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5µL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5µL/well

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 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.

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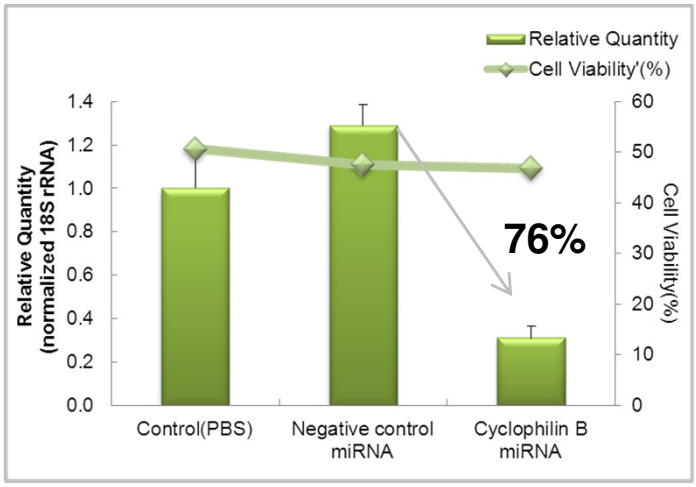
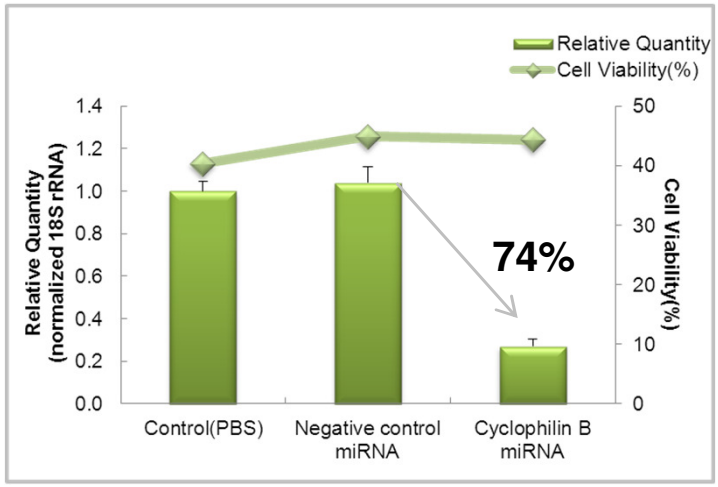
BALB/c mouse primary B cells

miRNA transfection

[1] Anti-CD40 / LPS stimulation



[2] Anti-CD40 / IL4 stimulation



Relative Quantity; qRT-PCR assays (n=3)
Cell Viability (%); PI staining assay

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

Isolated B cells → **Stimulation** **1 day** → Transfection → **No-stimulation** **2 days** → RT-PCR (Quantitation of mRNA)

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 (7 weeks old)]
【Stimulation】: [1] anti-CD40(1μg/mL)/LPS(1μg/mL), [2] anti-CD40(1μg/mL)/IL4(34.5ng/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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (2μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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.

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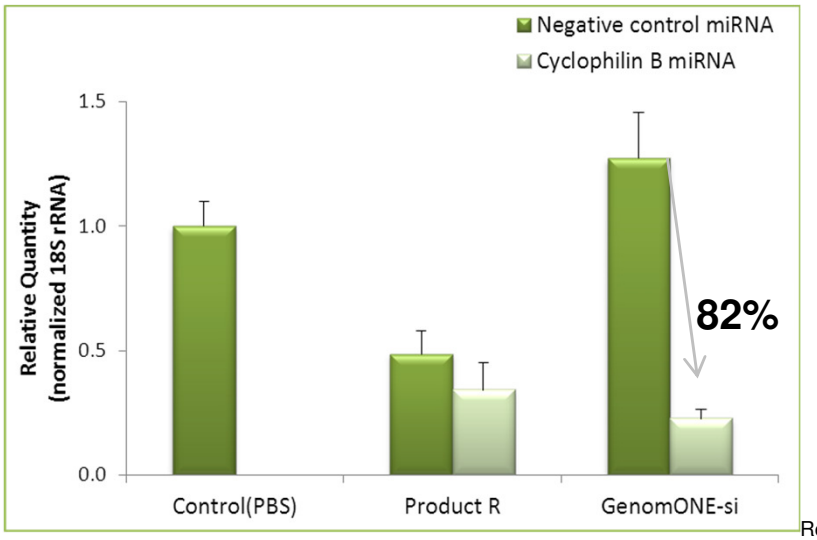
BALB/c mouse primary B cells

miRNA transfection



Relative Quantity; qRT-PCR assays (n=3)

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

Isolated B cells → **Stimulation** **1 day** → Transfection → **2 days** → RT-PCR (Quantitation of mRNA)

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 (8 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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (10μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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.

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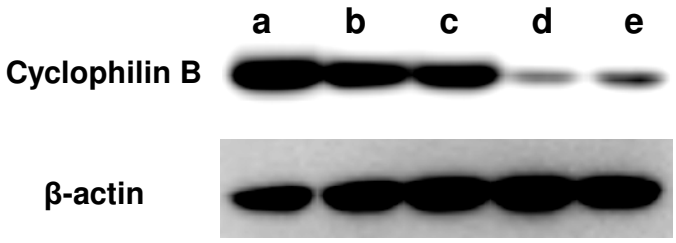
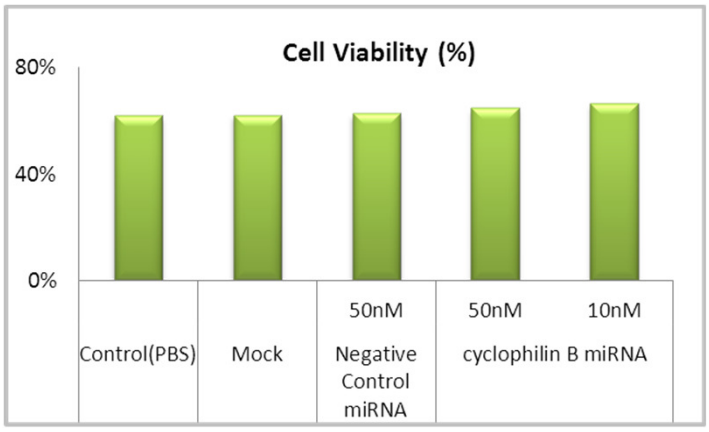
BALB/c mouse primary B cells

miRNA transfection



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



Cell Viability (%); PI staining assay

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

Isolated B cells → Stimulation (1 day) → Transfection → (2 days) → Western blotting

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 (11 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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (10~2μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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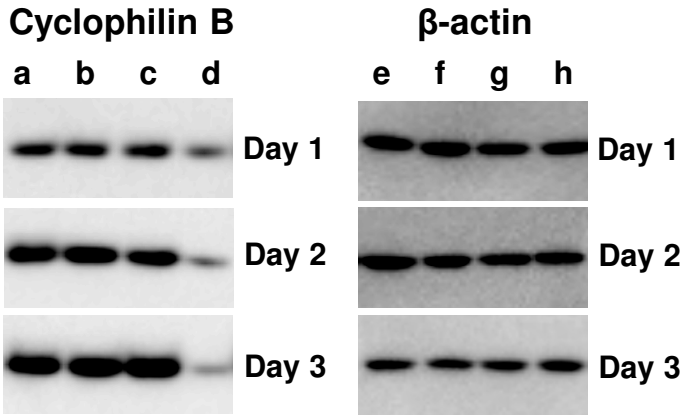
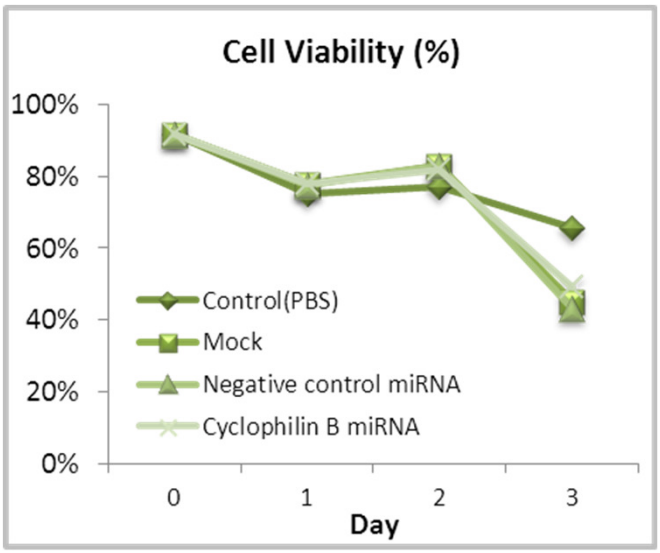
BALB/c mouse primary B cells

miRNA transfection



a, e: Control(PBS)
b, f: Mock
c, g: Negative control miRNA(50nM)
d, h: Cyclophilin B miRNA(50nM)



Cell Viability (%); PI staining assay

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

B cells isolation & Transfection → Stimulation 1～3 days → Western blotting

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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (30μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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 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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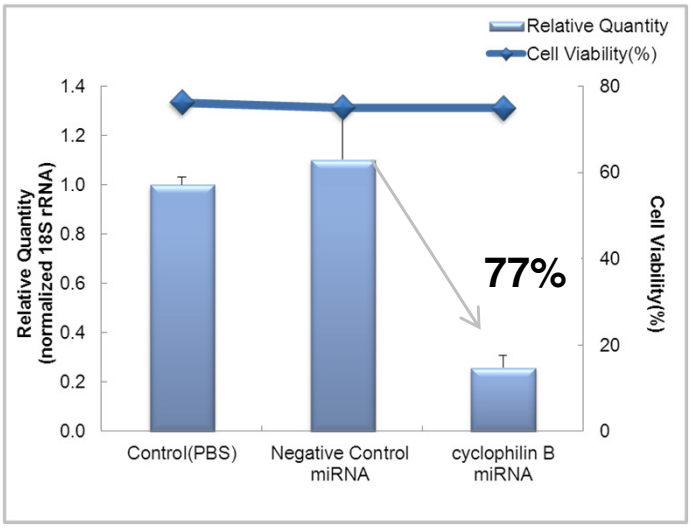
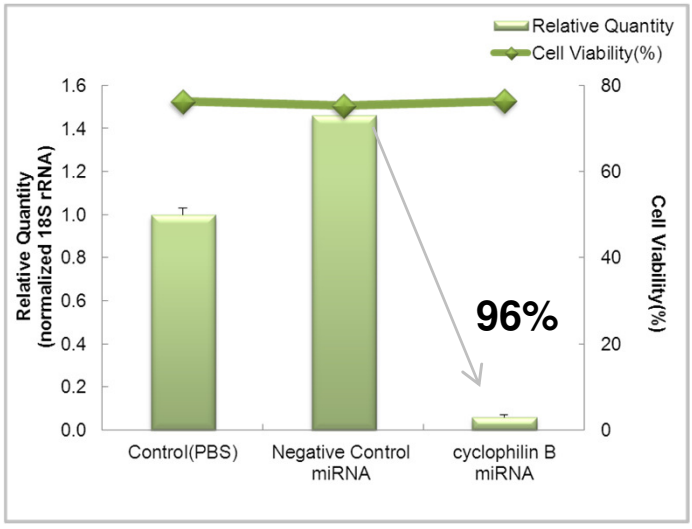
BALB/c mouse primary T cells

siRNA/miRNA transfection

siRNA transfection



miRNA transfection



Relative Quantity; qRT-PCR assays (n=2~3)
Cell Viability (%); PI staining assay

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

Splenocyte → (Stimulation, 1day) → T cells isolation & Transfection → (No-stimulation, 2days) → RT-PCR (Quantitation of mRNA)

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/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)
【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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA/miRNA solution and agitation (tapping) (30μM)	siRNA/miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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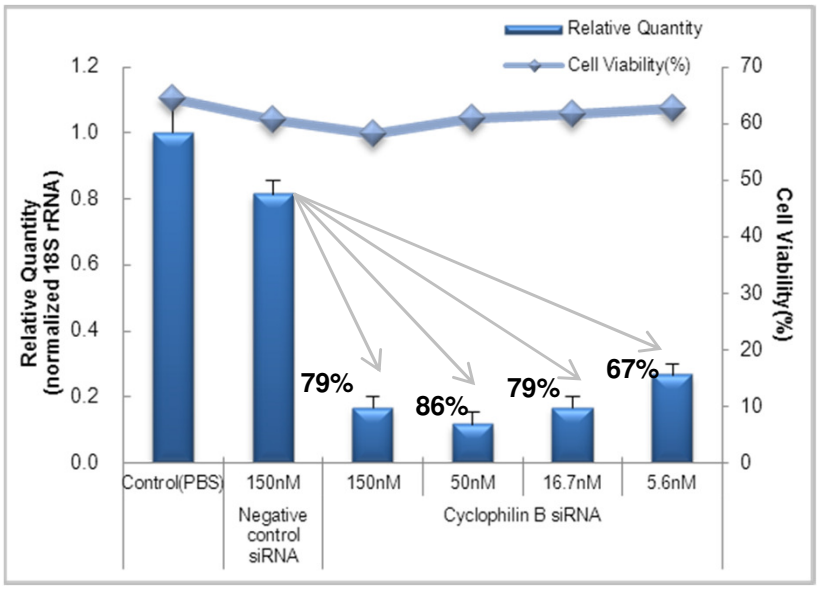
BALB/c mouse primary T cells

siRNA transfection



Relative Quantity; qRT-PCR assays (n=3)
Cell Viability (%); PI staining assay

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

Splenocyte → **Stimulation** **1 day** → T cells isolation & Transfection → **No-stimulation** **2 days** → RT-PCR (Quantitation of mRNA)

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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with siRNA solution and agitation (tapping) (30~1.1μM)	siRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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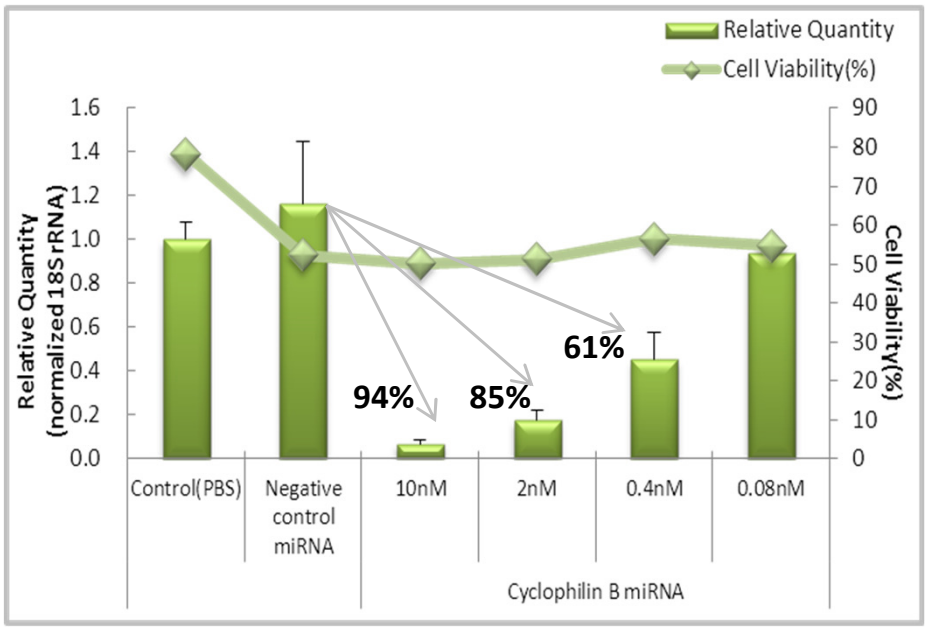
C57BL/6 mouse primary T cells

miRNA transfection



Relative Quantity; qRT-PCR assays (n=3)
Cell Viability (%); PI staining assay

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

Splenocyte → **Stimulation** **1 day** → T cells isolation & Transfection → **No-stimulation** **2 days** → RT-PCR (Quantitation of mRNA)

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 C57BL/6 mouse (6 weeks old)]
【Stimulation】: PMA(5nM) / ionomycin(1μg/mL)
【Culture condition】: 1×10^6 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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (2~0.016μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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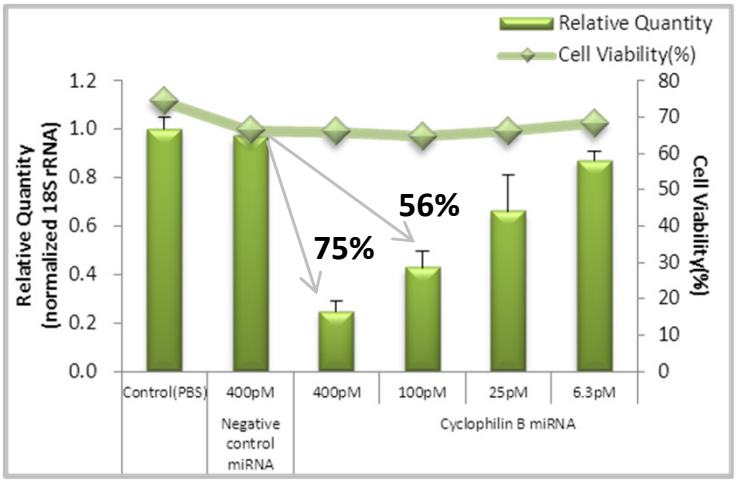
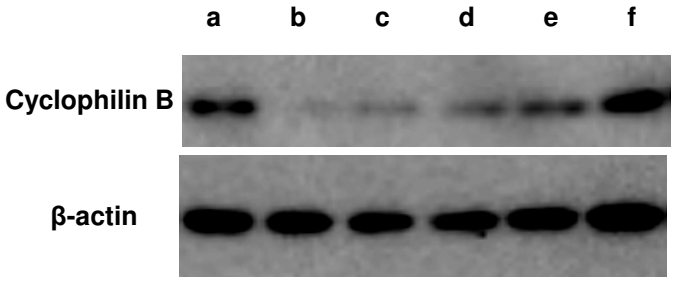
BALB/c mouse primary T cells

miRNA transfection



Relative Quantity; qRT-PCR assays (n=3)
Cell Viability (%); PI staining assay



a; Negative control miRNA (1000pM)
b; Cyclophilin B miRNA (1000pM)
c; Cyclophilin B miRNA (400pM)
d; Cyclophilin B miRNA (100pM)
e; Cyclophilin B miRNA (25pM)
f; Cyclophilin B miRNA (6.3pM)

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

Splenocyte → **Stimulation** **1 day** → T cells isolation & Transfection → **No-stimulation** **2 days** → ・RT-PCR (Quantitation of mRNA) ・Western blotting

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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (200~0.3nM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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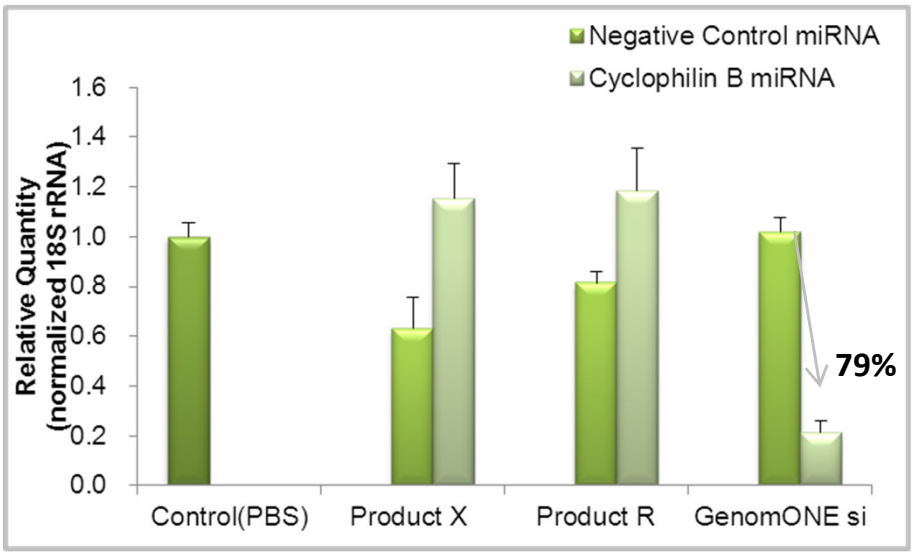
BALB/c mouse primary T cells

miRNA transfection



Relative Quantity; qRT-PCR assays (n=3)

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

Splenocyte → **Stimulation** **1 day** → T cells isolation & Transfection → **No-stimulation** **2 days** → RT-PCR (Quantitation of mRNA)

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 (9 weeks old)]
【Stimulation】: PMA(5nM) / ionomycin(1μg/mL)
【Culture condition】: 1×10^6 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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (2μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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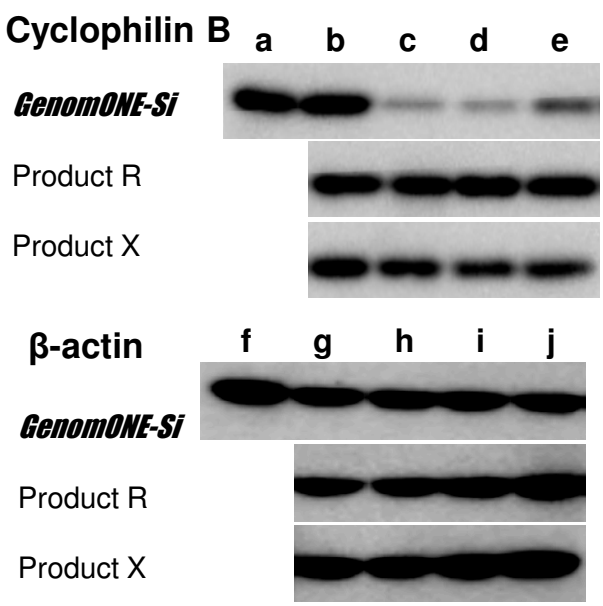
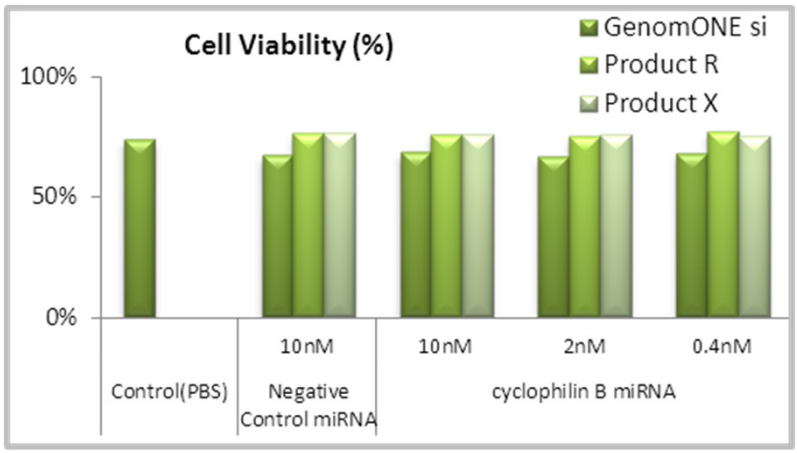
BALB/c mouse primary T cells

miRNA transfection





Cell Viability (%); PI staining assay

a, f: Control(PBS)
b, g: Negative control miRNA(50nM)
c, h: Cyclophilin B miRNA(50nM)
d, i: Cyclophilin B miRNA(2nM)
e, j: Cyclophilin B miRNA(0.4nM)

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

Splenocyte → **Stimulation** **1 day** → T cells isolation & Transfection → **No-stimulation** **2 days** → Western blotting

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 (8 weeks old)]
【Stimulation】: PMA(5nM) / ionomycin(1μg/mL)
【Culture condition】: 1×10^6 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)

	Step	Amount of reagent (24-well plate)
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E: 2.5μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL
(3)	Combination with miRNA solution and agitation (tapping) (2~0.08μM)	miRNA solution: 10μL
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well

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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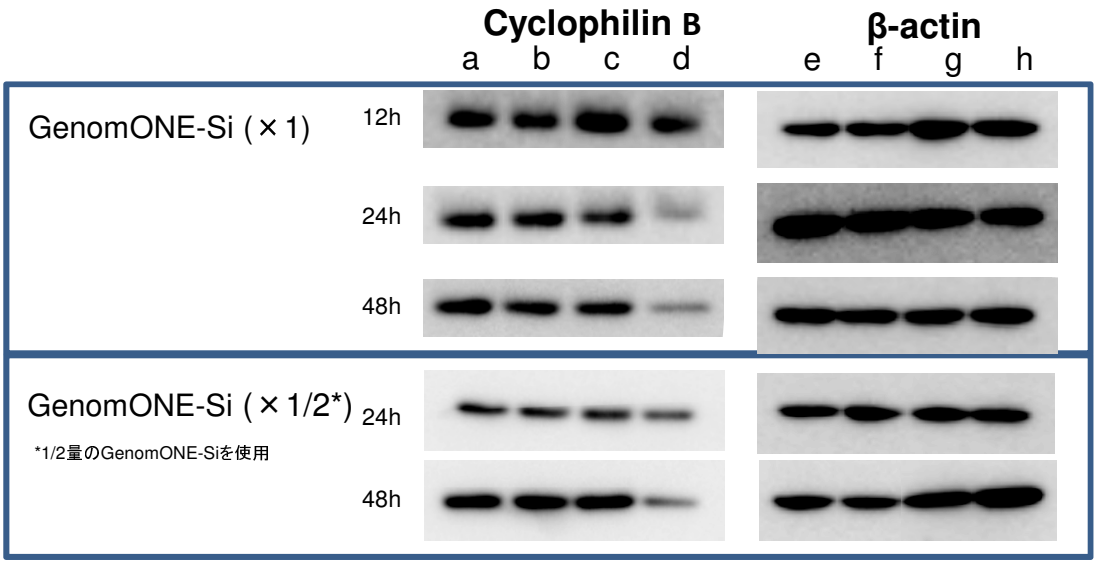
BALB/c mouse primary T cells

miRNA transfection



a, e: Control(PBS)　b, f: Mock　c, g: Negative control miRNA(50nM)　d, h: Cyclophilin B miRNA(50nM)

Transfection of Cyclophilin B miRNA intoPost-stimulated mouse primary T cells

T cells isolation & Transfection → Stimulation 12～48 h → Western blotting

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】: 1×10^6 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)

	Step	Amount of reagent（24-well plate）	
(1)	HVJ-E suspension taken into a micro-test tube	HVJ-E(×1): 2.5μL	HVJ-E(×1/2 vol.): 1.25μL
(2)	Combination with Reagent D and agitation (tapping)	Reagent D: 0.5μL	Reagent D: 0.25μL
(2)'	Combination with Buffer and agitation(tapping)	—	Buffer: 1.5μL
(3)	Combination with miRNA solution and agitation (tapping) (10μM)	miRNA solution: 10μL	
(4)	Combination with Reagent E and agitation (tapping)	Reagent E: 5μL	2-fold dilutions of Reagent E: 5μL
(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	Suspension[(1)+(2)+(3)+(4)]: 4.5μL/well	

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.

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