Wako Product Update

No. 15

Cellbiology
- Fluorescence
- Chemi-luminescence
- Protein Phosphorylation
- Protein Electrophoresis

Molecular Biology
- Nucleic Acid Electrophoresis
- CPG
- Transfection
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2006

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Please visit the Wako Online Catalog

http://www.e-reagent.com

Wako

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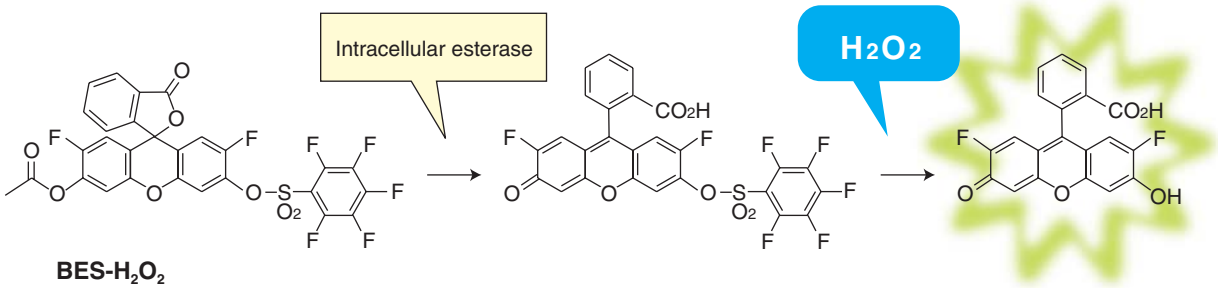
Alphabetical Index –Wako Product Update No.15–

Highly Selective Fluorescent Probe for Hydrogen Peroxide

BES-H_2O_2

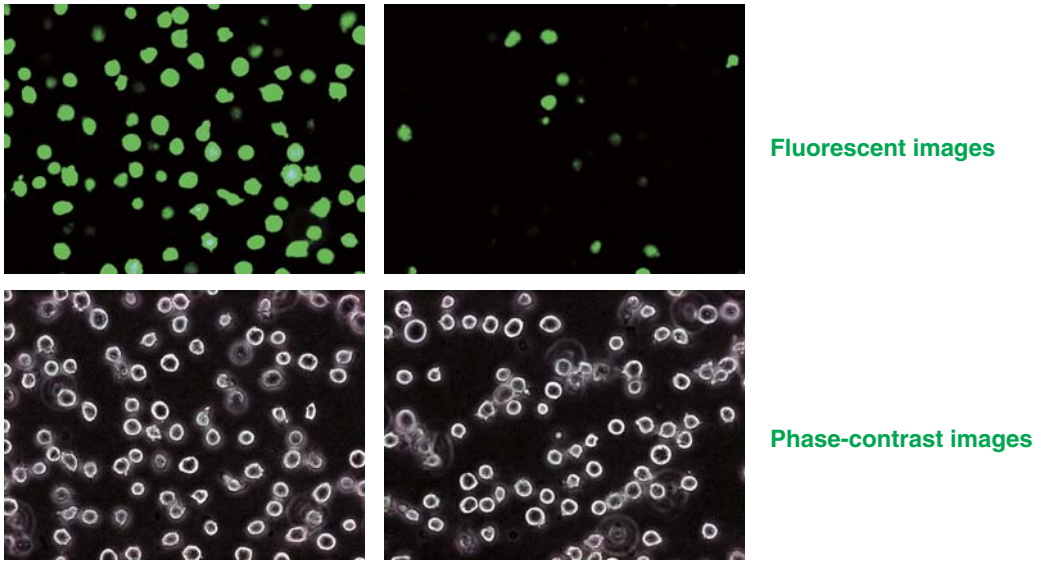
[Features]

1. Highly selectivity toward H_2O_2
2. Permeable to cell membrane
3. Detectability of cell-derived H_2O_2
4. Applicable to Molecular Imaging



BES-H_2O_2
[3'-*O*-Acetyl-6'-*O*-pentafluorobenzenesulfonyl-2',7'-difluorofluorescein]
$C_{28}H_{11}F_7O_8S$ = 640.44

Reactive oxygen species (ROS) such as superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO •) are important mediators of pathological processes in various diseases. 2',7'-Dichlorofluorescein (DCFH) and its diacetyl derivative have been widely used as fluorescent probes for measuring cell-derived H_2O_2, but these compounds suffer from the major drawback that they are poorly selective toward H_2O_2. Wako has launched BES-H_2O_2, which is a probe for cell-derived H_2O_2 with high selectively. It is applicable to clarifying cell response as well as dynamic function of H_2O_2 with diseases.



Fluorescent images of Jurkat T cells with BES- H_2O_2 and the same-field phase-contrast images
Jurkat T cells were cultured in a medium with 50μM BES-H_2O_2 for 1 hour. Then, one group of them was cultured in a medium with 5mM butyric acid (H_2O_2-produced stimulation), whereas the other in a medium without butyric acid (No H_2O_2-produced stimulation), each for 1 hour. (*Courtesy: Hatsuo Maeda, associate professor of Graduate School of Pharmaceutical Sciences, Osaka University, Japan*)

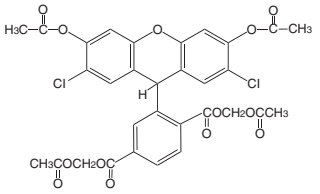
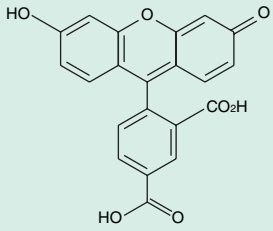
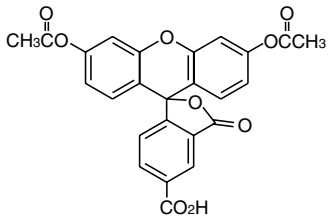
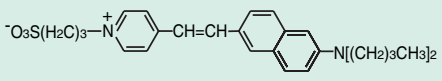
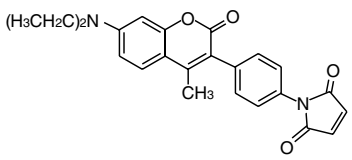
[References]

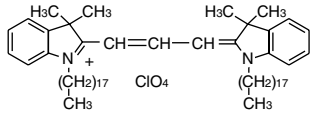
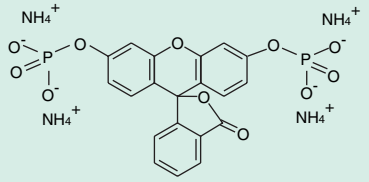
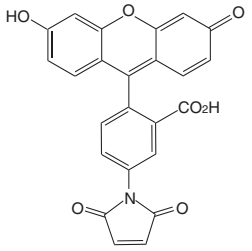
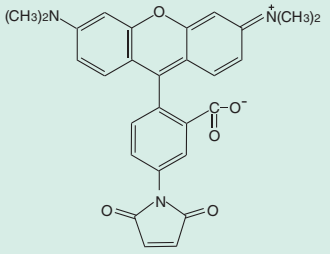
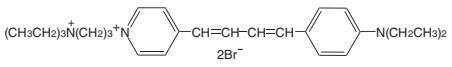
1) Maeda, H., Fukuyasu, Y., Yoshida, S., Fukuda, M. Saeki, K.. Matsuno, H., Yamauchi, Y., Yoshida, K., Hirata, K. and Miyamoto, K. : *Angew. Chem. Int. Ed.*, **43**, 239 (2004).
2) Maeda, H., Matsuura, S., Nishida, M., Senba, T.,Yamauchi, Y. and Ohmori, H. : *Chem. Pharm. Bull.*, **49**, 294 (2001).

Description	Cat. No. (Pkg. Size)	Solubility	Fluorescence
BES- H_2O_2, 94+% (HPLC)	029-15381 (1 mg)	Soluble in DMSO, DMF and acetonitrile	λex: 485 ± 20 nm; λem: 515 ± 20 nm

Fluorescent reagents

for Cellbioloogy

Fluorescent pH Indicator	**032-19331 (5 mg)**	**6-Carboxy-2′,7′-dichlorodihydrofluorescein Diacetate, Diaceloxymethyl Ester** λex = 291 nm, λem = none Solubility: Soluble in DMSO This product is membrane-permeant. It is subjected to the action of esterase and generates fluorescence. In addition, because it also produces fluorescence by undergoing oxidation, it is also used for the detection of free radicals, which are indicators of apotosis. The fluorescence spectra (λem) of this product after reaction is 529 nm.	 $C_{31}H_{24}Cl_2O_{13}$ = 675.42
for *in situ* labeling peptides, proteins and nucleotides	**039-19341 (100 mg)**	**5-Carboxyfluorescein [5-FAM]** λex = 492 nm (pH > 7.0), λem = 518 nm (pH > 7.0) Solvents for stock solutions: DMSO or DMF 5-FAM is a single isomer. It is one of the most popular green fluorescent reagents used for in situ labeling peptides, proteins and nucleotides. It has also been used to prepare various small fluorescent molecules. **[References]** 1) Hahn, M., *et al.: Electrophoresis*, **22**, 2691 (2001). 2) Avrahami, D., *et al.: Biochemistry*, **40**, 12591 (2001). 3) Hung, S. C., *et al.: Anal. Biochem.*, **243**, 15 (1996).	 CAS No. [76823-03-5]
Fluorescent pH Indicator	**038-19291 (100 mg)**	**5-Carboxyfluorescein Diacetate [5-CFDA]** λex = 492 nm, λem = 518 nm Solubility: Soluble in DMSO, MeOH, EtOH, Acetone 5-CFDA is membrane-permeant and thus can be loaded into cells via incubation. Once inside the cells, 5-CFDA is hydrolyzed by intracellular esterases to 5-carboxyfluorescein. **[References]** 1) Boitano, S., *et al.: J. Cell Sci.*, **98**, 343 (1991). 2) Goodall, H., *et al.: Nature*, **295**, 524 (1982). 3) Hansson, Y., *et al.: J. Immunol. Meth.*, **100**, 261 (1987). 4) Bruning, J. W., *et al.: J. Immunol. Meth.*, **33**, 33 (1980).	 $C_{25}H_{16}O_9$ = 460.39 CAS No. [79955-27-4]
Membrane Potential Dye	**041-29111 (5 mg)**	**Di-4-ANEPPS** **[Pyridinium,4-{2-[6-(dibutylamino)-2-naphthalenyl]ethenyl}-1-(3-sulfopropyl)-hydroxide, inner salt]** λex = 496nm, λem = 705nm Solubility: Soluble in DMF/DMSO/EtOH **[References]** 1) Davidenko, J.M., *et al. : Nature*, **355**, 349 (1992). 2) Fromherz, P., *et al. : Biochim. Biophys. Acta*, **1068**, 149 (1991).	 $C_{28}H_{36}N_2O_3S$ = 480.66 CAS No. [90134-00-2]
blue fluorescent thiol-reactive dye	**045-29131 (25 mg)**	**CPM,** 94+% (HPLC) [7-Diethyamino-3-(4-maleimidophenyl)-4-methylcou marin] Solubility: Soluble in DMSO This maleimide derivative of coumarin is essentially nonfluorescent until it reacts with thiols, making it possible to quantify thiols without a separation step. CPM is a good energy acceptor from tryptophan and a good energy donor to fluorescein. **[References]** 1) Grossman, H.S.: *Biochemistry*, **22**, 5369 (1983). 2) Zot, G.H., et al.: *J. Biol. Chem.*, **265**, 14796 (1990).	 $C_{24}H_{22}N_2O_4$ = 402.44 CAS No. [76877-33-3]

Lipophilic Carbocyanine Dye	048-29121 (50 mg)	**DiI [DiIC18(3), or 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine Perchlorate]** λex = 550 nm, λem = 565 nm Solubility: Soluble in DMSO/DMF/EtOH DiI is widely used carbocyanine membrane dye that labels cell membranes by inserting its two long (C18 carbon) hydrocarbon chains into the lipid bilayers. Particularly, it has been extensively used for the anterograde and retrograde labeling of neurons. [References] 1) Derzko,Z., *et al.: Biochemistry*, **19**, 6050 (1980). 2) Leuther, M.D., *et al.: J. Immunol.*, **127**, 893 (1981). 3) Honig, M.G. and Hume, R.I.: *Trends in Neurosci.*, **9**, 333 (1989). 4) McConnell, S.K., et al.: *Science*, **245**, 978 (1989).	 $C_{59}H_{97}ClN_2O_4$ = 933.87 CAS No. [41085-99-8]
Phosphatase fluorescent substrate	060-04521 (5 mg)	**Fluorescein Diphosphate Tetraammonium Salt [FDP]** λex = 272 nm, λem = none Widely used in ELISA assays and tyrosine phosphatase detection. The fluorescence spectra (λem) of this product after reaction is 514 nm. [References] 1) Yu, J.S., et al.: *J. Biochem.(Tokyo)*, **129**, 243 (2001). 2) Nolkrantz, K., *et al.: Anal. Chem.*, **74**, 4300 (2002). 3) Murakami, Y., *et al.: Biosens. Bioelectron*, **16**, 1009 (2001). 4) Ahumada, A. and Izquierdo, L.: *Biol. Res.*, **27**, 241 (1994). 5) Huang, Z., et al.: *J. Immunol. Methods*, **149**, 261 (1992).	 CAS No. [217305-49-2]
highly specific green fluorescent thiol-reactive dye	066-04501 (25 mg)	**Fluorescein-5-maleimide** λex = 492nm, λem = 515nm Solubility: Soluble in DMF, water (pH>6) [References] 1) Bigelow, J.D., *et al. : Biochemistry*. **30**, 2113 (1991).	 $C_{24}H_{13}NO_7$ = 427.36 CAS No. [75350-46-8]
fluorescent thiol-reactive dye	204-16131 (5 mg)	**Tetramethylrhodamine-5-maleimide** λex = 541 nm, λem = 569 nm Solubility: Soluble in DMSO This product is photostable and the fluorescence intensity is unaffected by the pH.	 $C_{28}H_{23}N_3O_5$ = 481.50 CAS No. [174568-67-3]
Membrane Potential Dye	141-07841 (5 mg)	**N-(3-Triethylammoniumpropyl)-4-{4-[4-(diethylamino)phenyl]butadienyl}pyridinium dibromide [RH 414]** λex = 532 nm, λem = 716 nm Solubility: Soluble in DMSO, EtOH This is a voltage sensitive probe that is similar to the Dialkylaminophenylpolyenylpyridinium dye. It undergoes changes in its electrical structure, which changes the fluorescence spectrum. This product is used to examine transient potential changes in excitable cells, including single neurons, cardiac cells and intact tissue preparations.	 $C_{28}H_{43}NBr_2N_3$ = 581.47 CAS No. [161433-30-3]

Green Chemiluminescent probe for superoxide anions

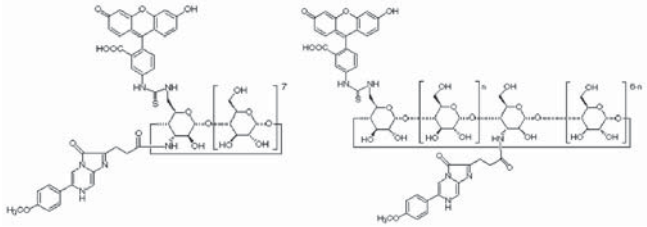
Green Chemiluminescent CD

Green Chemiluminescent CD is a highly sensitive chemiluminescence probe, which was developed by Dr. Teranishi of Mie University, Japan. This probe reacts with superoxide anion and produces luminescence at long wavelengths. Therefore the luminescence remains nearly unaffected by biomaterials. Additionally, this probe is more sensitive than other probes which produce luminescence at long wavelengths.

[Features]
1. **High luminescence intensity**
2. **Luminescence at long wavelengths (530 nm)**

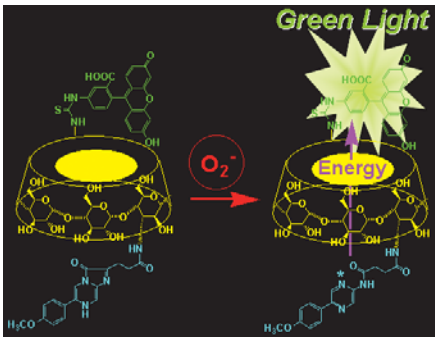
[Solubility]
- Soluble to hot methanol : H_2O(1:1) containing 0.1% TFA.

[Structure formula]



(1) (2) n=0~6
This product is mixture of 8 kinds of position isomers consisting of (1) and (2)

< Pathway of superoxide-induced chemiluminescence >



[References]
- Teranishi, K. and Nishiguchi, T. : *Anal. Biochem.*, **325**, 185 (2004).

Description	Wako Catalog No.(Pkg Size)	Storage
Green Chemiluminescent CD	075-05111(1 mg)	Keep at -20°C in the dark

3. Protein Phosphorylation

Highly potent, cell-permeable, selective ROCK inhibitor

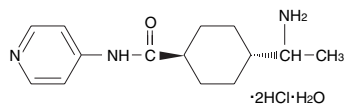
Y-27632

[(*R*)-(+)-*trans*-N-(4-Pyridyl)-40(1-aminoethyl)-cyclohexanecarboxamide • 2HCl • H_2O]

Y-27632 is a highly potent, cell-permeable and selective inhibitor of Rho-associated coiled coil forming protein kinases (ROCK). As well as modulating smooth-muscle contraction by inhibiting Ca^{2+} sensitization, Rho/ROCK pathway may help to regulate integrin-mediated cell adhesion and motility, which could be critical in processes such as tumour-cell metastass and immunoactivation. (Ki value of Y-27632 for $p160^{ROCK}$ = 140nmol/L)*

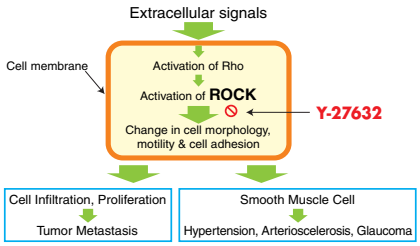
*: See the reference 1)

Solubility : H_2O (100mg/mL)



$C_{14}H_{21}N_3O \cdot 2HCl \cdot H_2O$ = 338.27
CAS: 146986-50-7

Signal Transduction of Rho/ROCK Pathway



[References]
1) Uehata, M. *et al.* : *Nature*, **389**, 990 (1997).
2) Sakamoto, K., *et al.* : *J. Pharmacol. Sci.*, **92**, 56 (2003).
3) Nishimaru, K., *et al.* : *J. Pharmacol. Sci.*, **92**, 424 (2003).

Description	Wako Catalog No. (Pkg Size)	Storage Condition
Y-27632, 94.0+% (HPLC)	257-00511(1 mg)	Keep at -20°C, protect from light Packaged under argon gas.

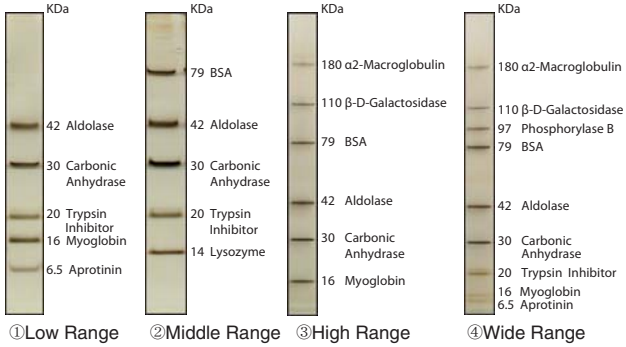
Sold under license of U.S. Patent 4,997,834 and PCT Patent WO98/66433A1 and under license from Mitsubishi Pharma Corporation.

4. Protein Electrophoresis

Silver Stain SDS-PAGE Molecular Weight Markers

Molecular weight markers applicable to silver staining are available. Each band is evenly stained because the protein bands are reduced and alkylated.

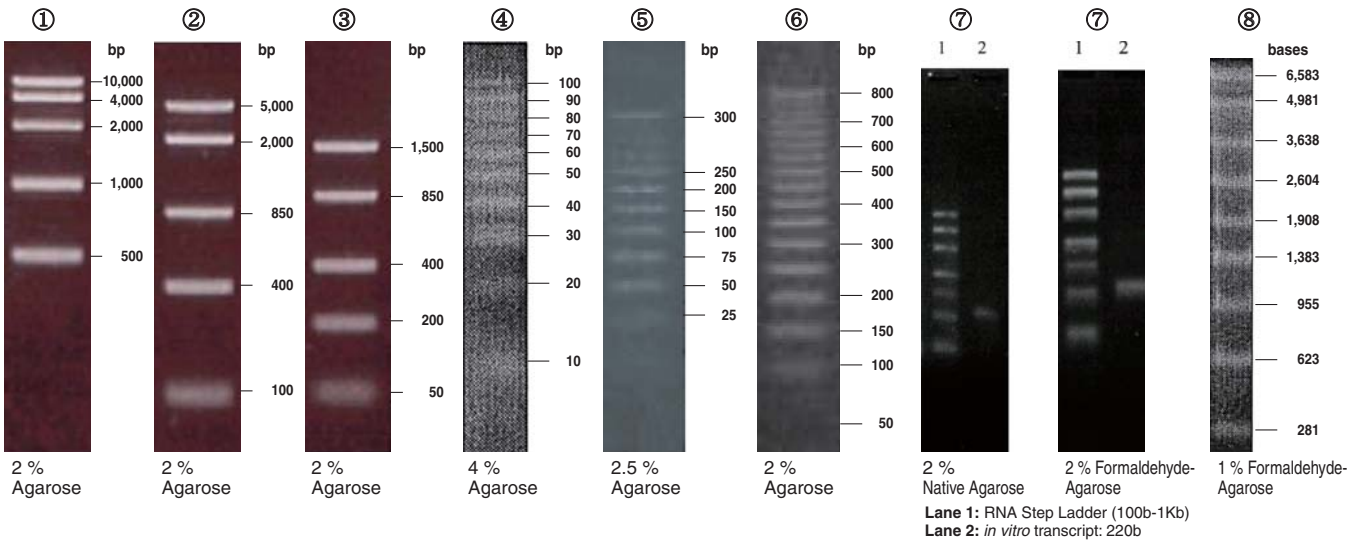
Fig.	Description		Wako Cat. No.	Package Size
①	Silver Stain MW Marker	Low Range	196-14001	for 6 mL
②		Middle Range	193-14011	for 6mL
③		High Range	190-14021	for 6mL
④		Wide Range	197-14031	for 6mL



①Low Range ②Middle Range ③High Range ④Wide Range

Each was run on a 5-20% SDS-PAGE gel Supersep® HG (Wako Cat. #195-13611) and silver stained with SilveraStain II Kit (Wako Cat. # 291-50301)

Fig.	Description	fragment range	Wako Cat. No.	Package Size	Size	Kit Contents	Storage
	DNA Step Ladder						
①	DNA Step Ladder *<15 min-separation>*	500-10,000 bp	292-61201	2 × 0.5 mL	500bp, 1k, 2k, 4k, 10kbp	1) DNA Step Ladder : 2 vials × 0.5mL 2) 6 × Loding Dye Solution : 1 vial	Keep at –20˚C
②	DNA Step Ladder *<15 min-separation>*	100-5,000 bp	296-61101	2 × 0.5 mL	100, 400, 850bp, 2k, 5kbp	1) DNA Step Ladder: 2 vials × 0.5mL 2) 6 × Loding Dye Solution : 1 vial	
③	DNA Step Ladder *<15 min-separation>*	50-1,500 bp	290-61001	2 × 0.5 mL	50, 200, 400, 850, 1500bp	1) DNA Step Ladder : 2 vials 2) 6 × Loding Dye Solution : 1 vial	
④	10 bp DNA Step Ladder	10-100 bp	040-28721	32.5 μg (50 μL)	10, 20, 30, 40, 50, 60 ,70, 80, 90, 100bp	1) DNA Step Ladder (10-100bp) : 1 vial 2) 6 × Blue/Orange Loading Dye : 1 vial	
⑤	25 bp DNA Step Ladder	25-300 bp	547-02301	90 μg (250 μL)	25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300bp	1) DNA Step Ladder (25-300bp) : 1 vial 2) 6 × Blue/Orange Loading Dye	
⑥	50 bp DNA Step Ladder	50-800 bp	544-02311	85 μg (250 μL)	50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800bp	1) DNA Step Ladder (50-800bp) : å 1 vial 2) 6 × Blue/Orange Loading Dye	
	100 bp DNA Step Ladder	100bp-1.5 kbp	546-01651	30 μg	100, 200, 286, 400, 500, 600, 717, 800, 900bp, 1k, 1.5kbp	1) DNA Step Ladder (100-1.5kbp) : 1 vial 2) 6 × Blue/Orange Loading Dye	
	DNA Step Ladder Mix	80-10,000 bp	544-02051	1 mL	80, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1031bp, 1.5k, 2k, 2.5k, 3k, 4k, 5k, 6k, 8k, 10kbp	1) DNA Step Ladder Mix 2) 6 × Blue/Orange Loading Dye	
	RNA Step Ladder						
⑦	RNA Step Ladder	100 b-1 kb	298-61301	5 × 0.04 mL	100, 200, 300, 400, 600, 800, 1000b	1) RNA Step Ladder : 5 vials 2) 2 × Loding Dye Solution : 1 vial	Keep at –80˚C
	RNA Size Standared Marker III	100b-1kb	545-01621	50 μg	100, 200, 300, 400, 500, 750, 1000b	—	Keep at –70˚C
⑧	RNA Size Standard Marker IV	0.28-6.58 kb	188-01831	50 μL	281, 623, 955, 1383, 1908, 2604, 3638, 4981, 6583b	—	Keep at –80˚C
	RNA Size Standared Marker II	0.5-9 kb	542-00651	50 μg	0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9kb	—	
	Ribosomal RNA Marker	16S + 23S	548-01731	2.5 mg	1776, 3556b	—	Keep at –70 ˚C
	Ribosomal RNA Marker	18S + 28S	545-01741	250 μg	2, 5.3kb	—	



Lane 1: RNA Step Ladder (100b-1Kb)
Lane 2: *in vitro* transcript: 220b

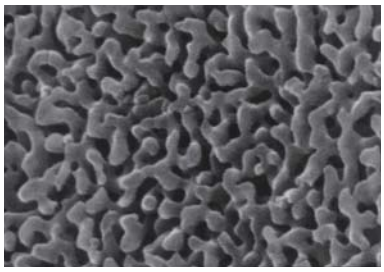
Solid-Phase Support for DNA Synthesis Genoglass-PG series

Genoglass-PG is a new pore glass developed by Genoglass. The Genoglass-PG, created using phase separation of borosilicate glass, has significantly improved the downfall of the existing controlled pore glass (CPG), which were the characteristic variations produced by the uneven pore diameters. In particular, in terms of DNA synthesis, Genoglass-PG has proven to reduce synthesis errors and improve yields by reducing steric hindrance, allowing for stable coupling efficiency. DNA synthesis supports (dN-Genoglass) with 4 types of First Nucleotides having different pore sizes, as well as γ-Amino-Genoglass for the synthesis of various compounds.

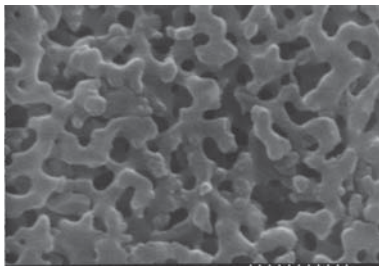
[Features]

1. **Excellent in terms of the evenness of the pores**
2. **Improved DNA synthesis yields**
3. **Low cost, and possibility of stable supply**

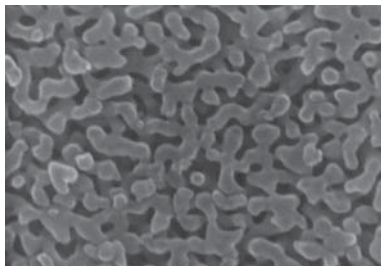
[Comparisons with scanning electron microscope]



Genoglass product:
Even surface and excellent porosity



Company P product:
Rough surface and fine pores uneven



Company C product: Low porositiy

Description	Wako Catalog No.		Binding Nucleoside	Pore Size	Specific Surface Area	Particle Size	Porosity	Surface Characteristics
	1 g	5 g						
DNA Synthesis supports [dN-Genoglass]								
Genoglass-PG-050-dA (Bz), 75-250	300-16561	306-16563	dA	500 Å	60~70 m^2/g	75~250 μm	0.8 mL/g ±5% (abt. 60 %)	Abt. 8 (μmol/m^2)
Genoglass-PG-100-dA (Bz), 75-250	308-16621	304-16623		1,000 Å	20~25m^2/g			
Genoglass-PG-050-dC (Bz), 75-250	307-16571	303-16573	dC	500 Å	60~70 m^2/g			
Genoglass-PG-100-dC (Bz), 75-250	305-16631	301-16633		1,000 Å	20~25 m^2/g			
Genoglass-PG-050-dG (ibu), 75-250	304-16581	300-16583	dG	500 Å	60~70 m^2/g			
Genoglass-PG-100-dG (ibu), 75-250	302-16641	308-16643		1,000 Å	20~25 m^2/g			
Genoglass-PG-050-dT, 75-250	301-16591	307-16593	dT	500 Å	60~70 m^2/g			
Genoglass-PG-100-dT, 75-250	309-16651	305-16653		1,000 Å	20~25 m^2/g			
γ-Amino-Genoglass								
Genoglass-PG-050-γ-Amino, 75-250	303-16551	309-16553	γ-Amino	500 Å	60~70 m^2/g			
Genoglass-PG-100-γ-Amino, 75-250	301-16611	307-16613		1,000 Å	20~25m^2/g			

• Please contact us for information on the type of dC (As) and 2,000 Å products, as well as solid-phase supports for RNA synthesis.
• Protect Group: Bz: benzoyl, ibu: isobutyl, Ac: actyl

One Step siRNA Transfection Plate

siScreen

ALLIANCE Technology,Inc.

With si*Screen* (siRNA pre-coated array plate) and your cells, transfection operation of siRNA completes. Without optimization of transfection condition, you can start siRNA knockdown experiments immediately. A wide variety of primary cells and hard-to-transfect cell lines are transfected without any instruments and devices.

[Features]

1. **Ready to Use & All in one plate**
   (Only plate cells, then transfection operation completes)
2. **Knockdown efficiency rises**
   (Comparison with the lipofection method -*see Fig 1*)
3. **With wide variety of cells, it realizes high transfection efficiency** *(see Fig 2)*
4. **High transfection on the reproducibility**
5. **Optimum to High-throughput experiments**
6. **Supports a wide range of platforms for your requests**

Fig. 1

Fig. 2

High knockdown efficiency

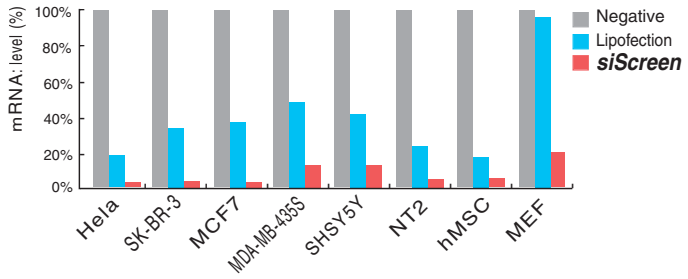
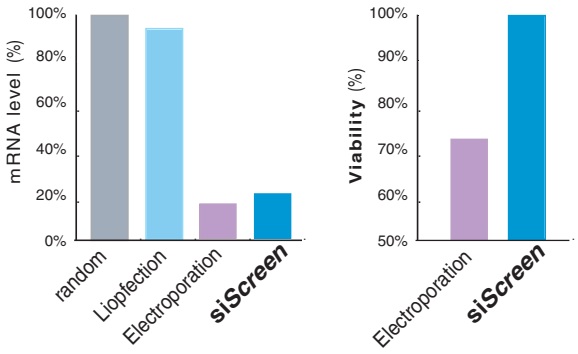


Fig1. It compared mRNA knockdown level by siScreen and the conventional Lipofection. As a result of Real-time PCR, siScreen decreased by more than 75 % of mRNA with any cells and they were more efficiently knockdown than Lipofection. Specifically, MEF (mouse ffibroblast) to be hardly knockdowned in Lipofecton, the difference is remarkable and the primary cell finds a valid in siScreen.

High cell viability

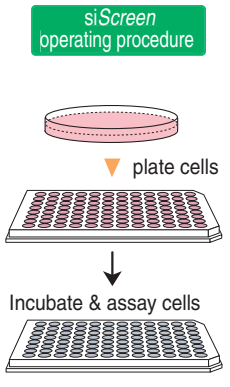


High transfection efficiency

Cells	Origins	KD level	Cells	Origins	KD level
Hela	Cervix carcinoma	97 %	HEK293	kidney	97 %
T-47D	breast cancer	94 %	HepG2	hepatic	98 %
SK-BR-3	breast cancer	96 %	NT2	embryonal cacinoma	94 %
MCF7	Breast carcinoma	97 %	SHSY5Y	neuro blastoma	85 %
MDA-MB-231	breast cancer	87 %	hMSC	Mesenchymal stem cell	94 %
MDA-MB-435S	breast cancer	86 %	MEF	mouse enbryonic fibloblast	77 %

Fig2. It compared the knockdown efficiency of mRNA with a wide range of cells. High knockdown efficiency is shown with all cells and si*Screen* supports an extensive cell species. It is possible to do a knockdown experiment immediately with a wide range of cells without transfection reagents and the optimization experiment of siRNA.

Ready-to-use & High Throughput Screening



- si*Screen* is All in One plate that is siRNA pre-coated in each plate well.
- Only plate cells, then siRNA transfection operation completes
- Without optimization of transfection conditions and experiments
- Optimum to High-throughput experiments

Description	Wako Cat. No.	ALLIANCE's product code	Package Size	
siScreen Trial	637-07593	SI 3011	1 plate × 24 well plate	Plate for test to confirm knockdown effect of siScreen with your cells
siScreen Apoptosis	632-07521	SI 1001	4 plates × 96 well plate	Precoated siRNAs to about 320 Human Apoptosis related genes and control siRNA in plate
siScreen Kinase	639-07531	SI 1002	1 plate × 96 well plate	Precoated siRNAs to 86 Human tyrosine kinase related genes and control siRNA in plate
siScreen Phosphatase	636-07541	SI 1003	3 plates × 96 well plate	Precoated siRNAs to about 200 Phosphatase related genes and control siRNA in plate
siScreen Custom 24	633-07551	SI 2024	6 plates × 24 well plate	Custom precoating service for your siRNAs. Free laying-out and available 24, 48, 96 or 384 well plate
siScreen Custom 48	630-07561	SI 2048	6 plates × 48 well plate	
siScreen Custom 96	637-07571	SI 2096	6 plates × 96 well plate	
siScreen Custom 384	634-07581	SI 2394	6 plates × 384 well plate	

for genome-wide screening!!

S. cerevisiae Direct Transformation Kit *Wako*

The *S. cerevisiae* Direct Transformation Kit *Wako* is specially designed for easy transformation of the budding yeast, *Saccharomyces* sp. The *S. cerevisiae* Direct Transformation Protocol allows successful transformation simply by mixing a plasmid DNA and the kit reagents with cultured yeast cells. No complicated steps, such as centrifugation or cell washing, are required. The *S. cerevisiae* Direct Transformation Kit *Wako* is particularly well suited for high-throughput transformation of a large number of yeast strains grown in 96-well plates. Even genome-wide screening, which has been problematic, becomes possible, since the *S. cerevisiae* Direct Transformation Kit *Wako* easily transforms about 4,850 strains with gene disruption.

[Features]

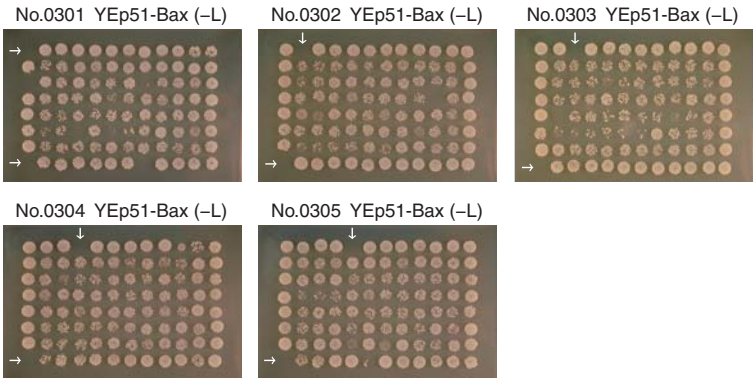
1. **No centrifugation or washing steps are required.**
2. **One-step procedure by mixing plasmid DNA and the kit reagents with cultured yeast cells.**
3. **Suitable for transformation of a large number of yeast strains using 96-well plates.**
4. **Applicable to tube protocol.**
5. **Low-viscosity reagent enables high-throughput screening.**

[Transformation Efficiency]

Transformation efficiency of BY4743 strain with pRS316 plasmid

	Transformation Efficiency (cfu/μg)
96-well plate protocol	≧ 500
Tube protocol	≧ 5,000

[Application with 96-well plates]



Plasmids were introduced into the strains of more than 95 %.
Strains that did not form colonies were affected by growth of mutants.
The colony formation of all strains was confirmed by reintroduction of plasmids.
A few cells (marked by arrows) remain blank to identify each plate.

(Data provided by: Dr. Akada, Associate Professor at the Faculty of Engineering, Yamaguchi University, Japan)

[Protocols]

<96-well plate protocol>

96-well plates
← YPD medium 25 μL/well
← **Inoculate yeast strains into each well**
Incubate the plate without shaking at 28-30 °C for 18-24 hours.
Suspend the cultured cell using a plate mixer.
← **plasmid/reagent solution** * 25 μL/well
Mix using a plate mixer
Incubate at 37 °C for 2 hours
Spot 5-10μL of the mixture
Incubate at 28-30 °C for 2-3 days
Transformed yeast

* : plasmid/reagent solution (per well, per tube)

Sc Transformation Reagent	20 μL
plasmid DNA	1 μg
Carrier DNA (5 μg/μL)	2 μL
Sterile water up to 25 μL	

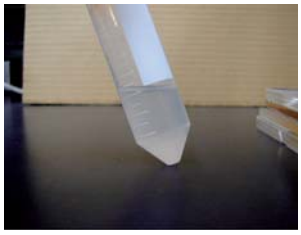
<Tube protocol>

Preparation of yeast culture
YPD medium 2 mL
← **Inoculate with a colony**
OD_{600} : Adjust to 0.01 with YPD medium
Transfer the 1 mL to a two-position type tube
Incubate with shaking at 28-30 °C until OD_{600} reaches 3.5-5.0
Yeast culture……①

Transformation
Microcentrifuge tube (1.5 mL)
← **plasmid/reagent solution** *25 μL and vortex
← **Yeast culture (①) 25 μL** and vortex
Incubate at 42 °C for 2 hours
← Sterile water 150 μL
Plating
Incubate at 28-30 °C for 2-3 days
Transformed yeast

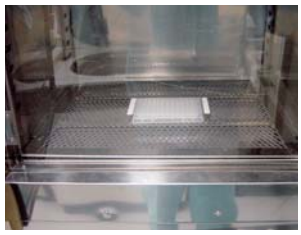
[96-well plate protocol]

Culture yeast cells in a 96-well plate. (YPD medium 25 μL/well)

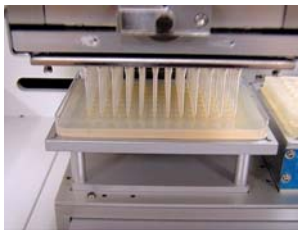


Prepare plasmid/reagent solution.

Add the plasmid/reagent solution into each well. (25 μL/well)



After mixing, seal the plate with microplate sealing tape and incubate at 37 °C for 2 hours.



Spot 10 μL of the mixture on an appropriate selection plate.

Incubate the selection plate at 28-30 °C for 2-3 days.

–*Application*– *Transformation efficiency : 11×0^5 cfu/μg!!*

<Realize higher transformation efficiency by addition of centrifugation procedure >

Preparation of yeast culture

YPD medium : 2 mL

← **Inoculate with a colony**

OD_{600} : Adjust to 0.01 with YPD medium

Transfer the 1mL of the suspension to a 14 mL two-position type tube

Incubate at 28-30 ℃ with shaking, until OD_{600} reaches 3.5-5.0

500 ×G, 5 min., RT

centrifugal concentration (×5)

Remove 800 μL of supernatant

Suspend by vortex mixer

Yeast culture suspension: 25 μL ← **Additional procedure**

Transformation

1.5 mL-microcentrifuge tube

← **Plasmid/reagent solution** * (25 μL) and vortex

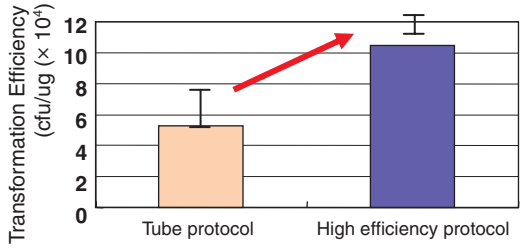
Incubate at 42 ℃ for 2hours.

Plating

Incubate at 28-30 ℃ for 2-3 days

Transformed yeast

[Data]



Description	Wako Cat. No.	Package Size	Storage Condition
***S. cerevisiae* Direct Transformation Kit** *Wako* Kit Contents : ① *Sc* Transformation Reagent ② Carrier DNA (5 μg/μL)	296-62701	20 tests	Keep at -20 ℃
	292-62703	100 tests	
	290-62704	500 tests	
[Related Products]			
Coming Soon! *S. pombe* Direct Transformation Kit *Wako*	290-64301	20 tests	
	296-64303	100 tests	
	294-64304	500 tests	

World's first cell-free protein synthesis system prepared from insect cells

Transdirect™ *insect cell*

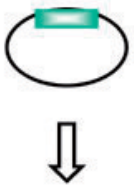
SHIMADZU BIOTECH bringing analysis to life

[Features]

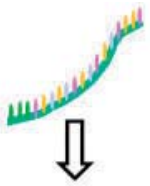
1. **Prepared from *Spodoptera frugiperda* 21 (Sf 21) insect cells**
   Sf21 insect cells are widely used in the baculovirus expression system.
2. **High protein synthesis efficiency**
   It is approximately 20 fold higher than that of rabbit reticulocyte cell-free system. 60–100μg of target protein can be synthesized per one kit. Furthermore, target protein can be acquired easily by affinity tag purification. The yield can be more than 20μg per one kit.
3. **Enables optical measurement due to clear and colorless reaction solution**
4. **Target protein is glycosylated by addition of canine microsomal membranes.**
5. **A multimer is synthesized with the arbitrary composition by regulating the density ratio of mRNA encoding different protein.**
6. **Expects analysis of posttranslational modification or protein interactions with MALDI-TOFMS**

[Kit Contents]

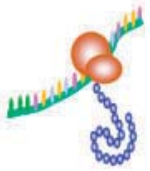
1.	Insect Cell Extract	(with yellow cap)	5 vials × 210 μL
2.	Reaction Buffer	(with blue cap)	1 vial × 630 μL
3.	4mM Methionine	(with red cap)	1 vial × 50 μL
4.	0.5 μg/μL pTD1 Vector (Expression Vector)	(with green cap)	1 vial × 10 μL
5.	0.5 μg/uL Control DNA (β-Gal)	(with white cap)	1 vial × 10 μL



Cloning with **pTD1 Vector** (expression vector), provided and your target gene



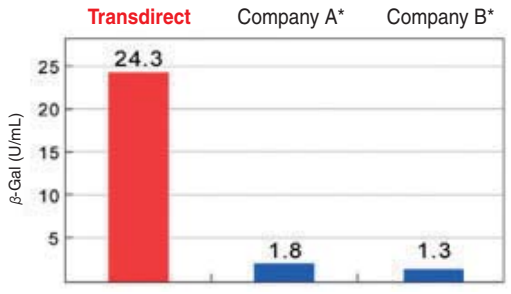
mRNA is synthesized and purified with commercial available kit and reagents.



in vitro translation with purified mRNA, **Insect Cell Extract**, provided, and **Reaction Buffer**, provided to get target protein

[Comparison Data on β-Gal (116 kDa) Synthesis]

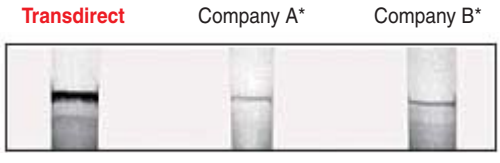
(A) Enzymatic activity



Company A and B in each table show data with other company's rabbit reticulocyte systems.

Measurement by β-Galactosidase Enzyme Assay System with Reporter Lysis buffer (Promega, E2000)

(B) Fluorescent detection



Detection by FluoroTect™ Green $_{Lys}$ *in vitro* Translation Labeling System (Promega, L5001)

Catalog No.	Description	Package Size	Kit contents	Storage
634-07601	**Transdirect™ *insect cell***	40 reactions	Insect Cell Extract/Reaction Buffer/4mM Methionine/pTD1 Vector/ Control DNA (β-gal)	Keep at –80 ℃

This product is developed and manufactured by SHIMADZU BIOTECH (Kyoto, Japan), and distributed by Wako. **http://www.shimadzu-biotech.jp/**

Please contact us for information on synthesis of protein including disulfide-bond formation.

Reagents for Molecular Biology, tested DNase, RNase and Protease activity

	Description	Wako Catalog No. (Package Size)	Activity Check
A	Adenosine 5′-Triphosphate Tetrasodium Solution	010-19531 (40 μmol)	DNase, RNase
	4-(2-Aminoethyl) benzenesulfonyl Fluoride Hydrochloride	019-20231 (100 mg); 015-20233 (1 g)	DNase, RNase
	2-Amino-2-hydroxymethyl-1, 3-propanediol [Tris]	019-20091 (100 g); 011-20095 (500 g); 015-20093 (1 kg)	DNase, RNase
	Ammonium Acetate	014-20482 (25 g); 018-20485 (500 g); 016-20481 (1 kg)	DNase, RNase
	Ammonium Peroxodisulfate	016-20501 (10 g); 012-20503 (100 g)	DNase, RNase
	Ammonium Sulfate	016-19871 (100 g); 018-19875 (500 g)	DNase, RNase
	Ampicillin Sodium	014-20161 (5 g); 010-20163 (10 g); 012-20162 (25 g)	DNase, RNase
B	Benzylsulfonyl Fluoride	022-15371 (1 g); 028-15373 (5 g); 020-15372 (25 g)	DNase, RNase
	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside [X-gal]	023-15041 (100 mg); 029-15043 (1 g)	DNase, RNase
	5-Bromo-4-chloro-3-indolyl-β-D-glucuronide Cyclohexylammonium Salt [X-Gluc]	025-15361 (10 mg); 021-15363 (100 mg)	DNase, RNase
	5-Bromo-4-chloro-3-indolylphosphate *p*-Toluidine Salt [BCIP *p*-Toluidine Salt]	026-15151 (100 mg); 022-15153 (500 mg)	DNase, RNase
C	Calcium Chloride Dihydrate	036-19731 (100 g); 038-19735 (500 g)	DNase, RNase
	Cesium Chloride	033-19682 (25 g); 035-19681 (100 g); 037-19685 (500 g)	DNase, RNase
	Chloramphenicol	032-19451 (5 g); 030-19452 (25 g); 038-19453 (100 g)	DNase, RNase
	Cytidine 5′-Triphosphate Tetrasodium Solution	030-18911 (40 μmol)	DNase, RNase
D	2′-Deoxyadenosine 5′-Triphosphate Sodium Salt Solution	041-29231 (20 μmol)	DNase, RNase, Phosphatase
	2′-Deoxycytidine 5′-Triphosphate Sodium Salt Solution	048-29241 (25 μmol)	DNase, RNase, Phosphatase
	2′-Deoxyguanosine 5′-Triphosphate Sodium Salt Solution	045-29251 (25 μmol)	DNase, RNase, Phosphatase
	Deoxyribonuclease I, Bovine, recombinant, Solution	548-02331 (1,000 U); 544-02333 (10,000 U)	RNase, Protease
	Deoxyribonucleoside 5′-Triphosphate Sodium Salt Mixture Solution	043-29291 (0.2 mL)	DNase, RNase, Phosphatase
	Deoxyribonucleoside 5′-Triphosphate Sodium Salt Solution	294-60801 (4 × 25 μmol)	DNase, RNase, Phosphatase
	2′-Deoxythymidine 5′-Triphosphate Sodium Salt Solution	042-29261 (25 μmol)	DNase, RNase, Phosphatase
	2′-Deoxyuridine 5′-Triphosphate Sodium Salt Solution	049-29271 (25 μmol)	DNase, RNase, Phosphatase
	N,N-Dimethylformamide	045-29192 (25 mL); 047-29191 (100 mL); 049-29195 (500 mL)	DNase, RNase
	Dimethyl Sulfoxide	041-29351 (50 mL); 047-29353 (100 mL); 043-29355 (500 mL)	DNase, RNase
	(±)-Dithiothreitol	044-29221 (100 mg); 040-29223 (1 g); 048-29224 (5 g); 042-29222 (25 g)	DNase, RNase
E	Ethanol (99.5)	052-07221 (100 mL); 054-07225 (500 mL)	DNase, RNase
G	D(+)-Galactosamine Hydrochloride	079-05011 (100 mg); 075-05013 (1 g); 073-05014 (5 g)	DNase, RNase
	50 mg/ml Geneticin® Disulfate Solution	071-04971 (20 mL); 077-04973 (100 mL)	DNase, RNase
	Gentamicin Sulfate	078-04981 (250 mg); 074-04983 (1 g); 072-04984 (5 g)	DNase, RNase
	Glycerol	070-04941 (100 mL); 072-04945 (500 mL)	DNase, RNase
	Glycogen Solution (abt. 20 mg/mL), from Mussel	079-05131 (1 mL)	DNase, RNase
	Guanidine Hydrochloride	072-05001 (100 g); 074-05005 (500 g); 078-05003 (1 kg)	DNase, RNase
	Guanidine Thiocyanate	073-04992 (25 g); 075-04991 (100 g); 077-04995 (500 g)	DNase, RNase
	Guanosine 5′-Triphosphate Tetrasodium Solution	075-04871 (40 μmol)	DNase, RNase
I	Isopropyl-β-D(-)-thiogalactopyranoside [IPTG]	090-05141 (100 mg); 096-05143 (1 g)	DNase, RNase
L	Lithium Chloride	121-05242 (25 g); 123-05241 (100 g); 129-05243 (500 g)	DNase, RNase
	D-Luciferin Potassium Salt *[a substrate to produce bioluminescence upon oxidation by firefly luciferase]*	126-05111 (10 mg); 122-05113 (25 mg); 120-05114 (100 mg); 126-05116 (1 g)	DNase, RNase
M	Magnesium Chloride Hexahydrate	131-15052 (25 g); 133-15051 (100 g); 135-15055 (500 g)	DNase, RNase
	2-Mercaptoethanol, 99%	131-14572 (25 mL); 133-14571 (100 mL)	DNase, RNase
P	Polyoxyethylene(20) Cetyl Ether [Brij 58]	162-21313 (5 g); 164-21312 (25 g)	DNase, RNase
	Polyoxyethylene(23) Lauryl Ether [Brij 35]	164-21611 (100 g); 166-21615 (500 g)	DNase, RNase
	Polyoxyethylene(10) Octylphenyl Ether [Triton X-100]	163-21201 (100 mL); 165-21205 (500 mL)	DNase, RNase
	Polyoxyethylene(20) Sorbitan Monolaurate [Tween 20]	160-21211 (50 g); 166-21213 (100 g)	DNase, RNase
	Polyoxyethylene(20) Sorbitan Monooleate [Tween 80]	161-21621 (50 mL); 163-21625 (500 mL)	DNase, RNase
	Potassium Acetate	164-21552 (25 g); 166-21551 (100 g); 168-21555 (500 g)	DNase, RNase
	Potassium Chloride	166-22112 (25 g); 160-22115 (500 g); 168-22111 (1 kg)	DNase, RNase
	2-Propanol	166-21671 (100 mL); 168-21675 (500 mL)	DNase, RNase
	Proteinase K Solution	166-21051 (5 mL)	DNase, RNase
R	Ribonucleoside 5'-Triphosphate Sodium Salt Mixture Solution	183-02001 (1 mL)	DNase, RNase, phosphatase
S	Sodium Acetate	191-13912 (25 g); 195-13915 (500 g); 193-13911 (1 kg)	DNase, RNase
	Sodium Chloride	192-13925 (500 g); 190-13921 (1 kg)	DNase, RNase
	Sodium Cholate	198-13721 (5 g); 196-13722 (25 g)	DNase, RNase
	Sodium Dextran Sulfate 5000	194-13402 (25 g); 196-13401 (100 g); 198-13405 (500 g)	DNase, RNase
	Sodium Dodecyl Sulfate	190-13982 (25 g); 192-13981 (100 g); 194-13985 (500 g)	DNase, RNase
	Spermidine	191-13831 (1 g); 197-13833 (5 g)	DNase, RNase
	Sucrose, Ultra Pure	198-13525 (500 g); 196-13521 (1 kg)	DNase, RNase
T	100 w/v% Trichloroacetic Acid Solution	204-16212 (25 mL); 206-16211 (100 mL); 208-16215 (500 mL)	DNase, RNase
U	Urea	215-01211 (100 g); 217-01215 (500 g); 211-01213 (1 kg)	DNase, RNase
	Uridine 5′-Triphosphate Tetrasodium Solution	212-01101 (40 μmol)	DNase, RNase

World's First New Subtraction Kit Using Enzyme Digestion

DsDD cDNA Subtraction kit *wako*

From Physical Absorption Method to DsDD

The subtraction hybridization method is a powerful technique used to identify genes that are specifically expressed in tissue, cell type or at a specific stage. Several methods, including physical absorption, have been used. Traditional procedures often had several drawbacks such as being complex and labor-intensive, time-consuming, and technically demanding. They required several rounds of hybridization and took about 4 days. They also required the Tester and Driver cDNA to be prepared each time from mRNA because the cDNA Library could not be used as the starting material due to the influence of vector-derived sequences.

The DsDD cDNA Subtraction Kit Wako (patent pending) is a method based on "Duplex-specific Direct Digestion" (DsDD), which overcomes traditional subtraction methods. The Tester and Driver cDNA form drawbacks of hybrids of genes, that are expressed nonspecifically. After hybridization, the hybrid ds cDNA from Tester and Driver cDNA are digested by duplex-specific nuclease. Finally, the Driver cDNA is removed by Exonuclease. This method efficiently enriches cDNA specifically expressed in Tester cDNA.
For example, when analyzing specific functions and properties of cancer cell tissue, the Tester is prepared from cancer tissue and the Driver from normal tissue, then cDNA derived from cancer specified gene is enriched. The DsDD cDNA Subtraction Kit Wako is a revolutionary technique that uses the cDNA Library as starting material, and the subtracted Tester cDNA can be recovered intact.

[Features]

1. **Start with established cDNA Libraries**
2. **Intact recovery of the subtracted Tester cDNA**
3. **Adoption of duplex-specific nuclease digestion**
4. **2 day performance**

What is Subtraction?

Example)

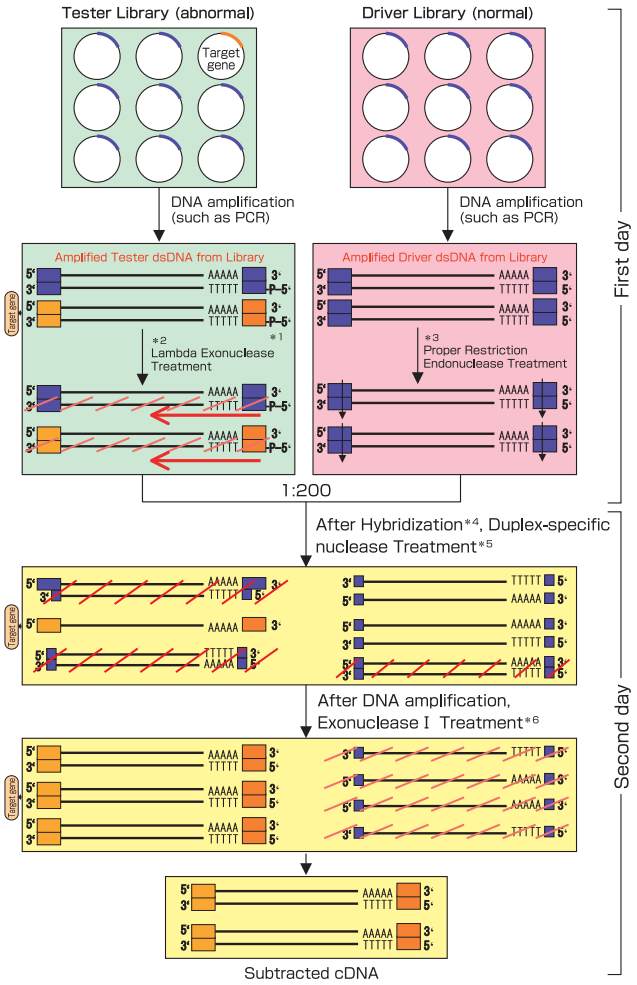
Tester		Driver
Hepatoma cells / tissues		Normal liver cells / tissues
A. Housekeeping genes		A. Housekeeping genes
B. Liver-specific genes	−	B. Liver-specific genes
C. Hepatoma-specific genes		

(A,B,C)−(A,B)= **C**

Subtraction is an efficient gene analysis method that enriches the amount of differentially expressed genes by subtracting the expressed genes present in both the Driver cDNA and the Tester cDNA.


Duplex-specific nuclease is purified from Kamchatka crab hepatopancreas

[Principle]



A Tester cDNA Library and a Driver cDNA Library are prepared. In the cDNA Library for the Tester, the cDNA should be inserted in the same direction as the vector.

∗1 Tester cDNA Amplification
During DNA amplification, a primer with phosphoric acid added to the 5' end is used.

∗2 Lambda Exonuclease Treatment
The double-stranded DNA's 5' phosphorylated primer is recognized and is degraded by a 5' to a 3'. Subtraction efficiency is enhanced as there is no hybridization of Testers.

∗3 Restriction Endonuclease Treatment
The digestion of adapters on both ends ensures accurate hybridization results.

∗4 Hybridization
The Tester cDNA and Driver cDNA react together at 68 C for 16 to 20 hours (mixing ratio =1:200) Most of the Tester cDNA form ds DNA due to the excessive amounts of Driver cDNA.

∗5 Duplex-specific nuclease Treatment
Enzymes that specifically cleave ds DNA are used. A high reaction temperature (68 C)makes reactions highly specific. After the reaction, only the single-stranded DNA containing the Tester' s specifically expressed genes will remain.

∗6 Exonuclease I Treatment
Enzymes that specifically cleave single-stranded DNA are used.
Non-specific genes are single stranded, thus degraded.

This highly efficient cDNA subtraction method gives results in only 2 days.

Application data

[Sample preparation from small amounts of total RNA using SMART method]

cDNA was prepared from 100 ng each of template total RNA of HepG2 (ATCC No. HB-8065) and normal liver tssue (BioChain Institute, Inc.) using the SMART PCR cDNA Synthesis Kit. Subtracted cDNA was prepared from Tester cDNA (GepG2) and Driver cDNA (normal liver) using the DsDD cDNA Subtraction Kit *Wako*. Subtraction efficiency was evaluated by electrophoresis and real-time PCR. GAPDH, which is a house-keeping gene, and AFP, which is specifically expressed gene in HepG2, were targeted as control genes.

<Preparation of Tester cDNA>

HepG2 Total RNA (100 ng)

↓

Reverse transcription — use of SMART PCR cDNA Synthesis Kit
The following primer with dT + PCR tag was designed because the use of the SMART PCR cDNA Synthesis Kit causes the 5' end and 3' end to have the same sequences.
5'-GCATCACTGATCGCACAGGTCGTCTACTTTTTTTTTTTTTTTTTVN-3' (47mer)

↓

PCR — PCR Primer II included in SMART PCR cDNA Synthesis Kit and newly synthesized primer; 5'-pGCATCACTGATCGCACAGGTC-3' (5' phosphorylated 21mer primer) were used.

↓

<Preparation of Driver cDNA>

Normal liver tissue Total RNA (100 ng)

↓

Reverse transcription — use of SMART PCR cDNA Synthesis Kit

↓

PCR

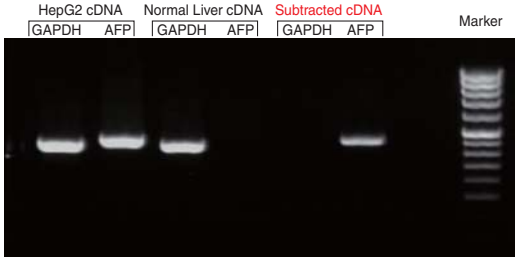
↓

Digestion by *Rsa*I (GT/AC)

↓

Prepared 1ng of Tester cDNA and 200ng of Driver cDNA were hybridized and subtraction was performed according to the protocol of the kit.

<Electrophoretogram>



In the subtracted cDNA, AFP genes, which are specifically expressed genes in HepG2, were confirmed but the highly expressed housekeeping GAPDH genes were not.

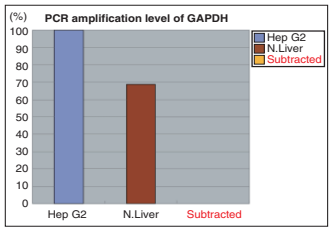
<Analysis by real-time PCR>

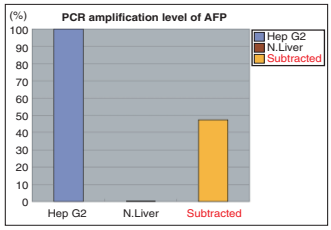
<GAPDH>

	Ct value	PCR amplification level (fg)
HepG2 cDNA	13.725	3677.09
Normal Liver cDNA	14.3	2526.41
Subtracted cDNA	28.09	0.31

<AFP>

	Ct value	PCR amplification level (fg)
HepG2 cDNA	12.27	4097
Normal Liver cDNA	20.51	15.28
Subtracted cDNA	14.125	1934.86

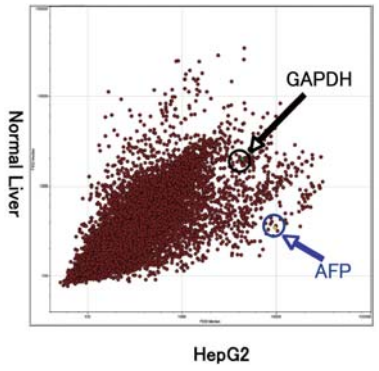


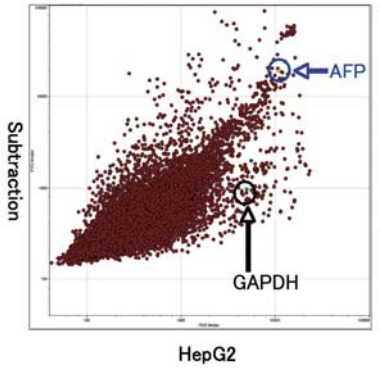


PCR amplification levels of GAPDH and AFP in the subtracted cDNA were less than about 1/10,000 and about 1/2 of those in the HepG2 cDNA, respectively, indicating that the subtraction was performed efficiently.

[More effective gene analyses by combination use with DNA chip]

Specifically expressed gene can be analyzed with low background by hybridizing Tester cDNA, concentrated using the DsDD cDNA Subtraction Kit Wako, to an array.





<Reaction and analysis condition>

Microarray: AceGene chip 30k human
(HITACHI Software Engineering Co., Ltd.)
Amplification by T7 polymerase reaction
Template used 5 μg
Labeled Cy3 and Cy5 (reaction for 40 °C, 1 hr.)

Microarray hybridization time=18 hr.
Microarray hybridization temperature=45 °C
Wash protocol
2 × SSC /0.5% SDS 5 min.
2 × SSC 5 min.
1 × SSC 5 min.
0.5 × SSC dip

↓

Scanning of Microarray chip (TIFF image DATA)
Analysis software: GenePix Pro 6.0

The housekeeping gene (GAPDH) is sufficiently subtracted and the fluorescence signal of the specifically expressed gene (AFP) is further enhanced (concentrated). This is a great advantage in gene cloning and it is considered to be able to extract characteristic genes. However, some ribosomal genes are also simultaneously concentrated in this method. Cancer tissues / cancer cells specific genes can be searched more specifically by preparing a Driver cDNA using mixed cancer cell lines to eliminate ribosomal genes.

[Kit Contents (for 5 reactions)]

1.	Lambda Exonuclease	5 μL × 1 tube
2.	10 × Lambda Exonuclease Buffer	25 μL × 1 tube
3.	4 × Hybridization Buffer	25 μL × 1 tube
4.	Duplex-specific nuclease	5 μL × 1 tube
5.	2 × Duplex-specific nuclease Buffer	25 μL × 1 tube
6.	Exonuclease I	5 μL × 1 tube
7.	10 × Exonuclease I Buffer	25 μL × 1 tube
8.	Ethachinmate	50 μL × 1 tube
9.	Stop Solution	50 μL × 1 tube
10.	3 mol/L Sodium Acetate, pH 5. 2	200 μL × 1 tube

[Storage Condition]

–20℃

[Product]

Product Name	Catalog No.	Package Size
DsDD cDNA Subtraction Kit *Wako*	294-62001	for 5 reactions

Related Products

Product Name		Catalog No.	Package Size
Lambda Exonuclease		290-61501	1,000 units
Exonuclease I		294-61401	4,000 units
dNTP Mixture		043-29291	0.2 mL
Phenol/ Chloroform/ Isoamyl Alcohol (25 : 24 : 1)	[NIPPON GENE CO., LTD.]	311-90151	250 mL
Ethanol (99.5)		052-07221	100 mL
		054-07225	500 mL
100bp DNA Step Ladder (100 – 1.5 kbp)	[Wako Chemicals USA]	546-01651	30 Ug
Agarose S	[NIPPON GENE CO., LTD.]	312-01193	100 Ug
50 × TAE	[NIPPON GENE CO., LTD.]	313-90035	500 mL
6 × Loading Buffer Triple Dye	[NIPPON GENE CO., LTD.]	314-90261	1mL × 3
Ethidium Bromide Solution	[NIPPON GENE CO., LTD.]	315-90051	10 mL
Distilled Water, Deionized, Sterile	[NIPPON GENE CO., LTD.]	316-90101	100 mL

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