

PRODUCT INFORMATION

Product Name: Alkaline Phosphatase (PAP)
(from *Shewanella sp.* SIB1)*

Code No: DE110

Lot No: 041013

Size: 1,000 unit

Concentration: 5unit/ μ l

Supplied reagent · 10 \times Alkaline Phosphatase Reaction Buffer
· Dilution Buffer

Storage: Store at -20

Source: *E.coli* harboring the plasmid encoding the gene of alkaline phosphatase from *Shewanella sp.* SIB1 (PAP).

Unit Definition: One unit is defined as the amount required to hydrolyzed 1.0 μ mole p-nitrophenyl phosphate per 1 minute in glycine/NaOH buffer at pH10.5 and 37 $^{\circ}$ C.

Assay conditions: The reaction mixture (100 μ l) contains 50mM glycine-NaOH buffer, pH 10.5, 5mM MgCl₂, 0.5mM ZnCl₂, 100mM KCl, 5mM p-nitrophenyl phosphate.

Storage Buffer: 10mM Tris-HCl pH7.5
0.025mM ZnCl₂
0.25mM MgCl₂
50% glycerol

Contaminants:

Dnase: When 0.5 μ g of *Hind III* digest was incubated with 10units of this enzyme in a 40 μ l reaction mixture for 18 hours at 37 $^{\circ}$ C, no degradation of the DNA fragment is observed on agarose gel electrophoresis.

BioDynamics Laboratory Inc.

PRODUCT INFORMATION

Rnase: No RNase activity is observed by the use of RnaseAlert assay (Ambion). In this assay the reaction mixture containing the fluorescent-labeled RNA substrate was incubated with 10units of this enzyme for 1 hours at 37 °C.

Composition of Supplied Reagent:

10 × Alkaline Phosphatase Reaction Buffer (Store at –20 °C)

1. 5M	Tris-HCl, pH7.3
125mM	glycine
0. 5%	TritonX-100
0.25mM	ZnCl ₂
2.5mM	MgCl ₂
60mM	NiCl ₂

Dilution Buffer (1 × Reaction Buffer, Store at –20 °C)

150mM	Tris-HCl, pH7.3
12.5mM	glycine
0.05%	TritonX-100
0.025mM	ZnCl ₂
0.25mM	MgCl ₂
6mM	NiCl ₂

* Licensed Under Japan Patent NO. 2001-172653

BioDynamics Laboratory Inc.

PRODUCT INFORMATION

Kit Manual

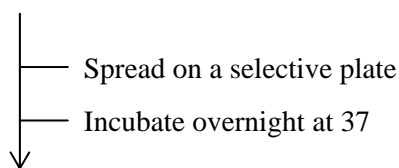
Dephosphorylation of 5' end by PAP

Linearized plasmid DNA * ¹	88μl (5' termini 10pmol)* ²
10 × Reaction buffer	10μl
Diluted PAP solution* ³	2μl* ⁴
<div>Incubate for 30 min at 60 (blunt end and 3'-protruding end) or at 37 (5'-protruding end)</div>	
<div>Inactivation by incubation for 5min at 95 *⁵</div>	
Dephosphorylated Plasmid DNA	

Ligation Reaction and Transformation

Dephosphorylated Plasmid DNA	1μl (0.05pmol)* ⁶
Insert DNA (foreign)	1μl (0.05 ~ 0.3pmol)* ⁷
10 × Ligation buffer* ⁸	1μl
T4 DNA ligase	0.5 Weiss unit
10mM ATP	1μl
H ₂ O	up to 10μl
<div>Incubate for 1 hour at 12 (blunt end) or at 16 (5'-protruding end and 3'-protruding end)</div>	
2μl (0.01pmol)* ⁹	
<div>← Add 100μl of competent cell</div>	
Store on ice for 30min	
Heat shock for 30sec at 42	
Store on ice 1min	
<div>← Add 0.9ml of SOC medium</div>	
Incubate for 1hr at 37	

PRODUCT INFORMATION



*1 Before the dephosphorylation, the complete digestion of the plasmid DNA should be confirmed by the agarose gel electrophoresis.

Restriction enzyme buffer (such as low buffer, medium buffer and high buffer) and 1×TE buffer is permissible as a buffer solution of the linearized plasmid DNA.

*2 Amount and length of the linearized plasmid DNA

Table 1

1kb linear DNA	10pmol of 5'-termini	=	3.3μg
2kb linear DNA	10pmol of 5'-termini	=	6.6μg
3kb linear DNA	10pmol of 5'-termini	=	9.9μg
4kb linear DNA	10pmol of 5'-termini	=	13.2μg

For example, 10pmole of 5'-termini of the linearized pUC18 (2.69kb) is 8.8μg.

*3 Amount of PAP

Amount of PAP should be modified depending on the kind of termini and the amount of 5'-termini of linearized plasmid DNA. The following amounts are recommended:

Table 2

Terminus		Units of PAP	
5'-Protruding	(10pmol)	1.0 units (37	30min)
Blunt	(10pmol)	2.5 units (60	30min)
3'-Protruding	(10pmol)	5.0 units (60	30min)

PRODUCT INFORMATION

PAP should be diluted with dilution buffer (1×reaction buffer) according to the table above. As the amount of PAP described in the table above are about ten times as much as that of the minimum effective amount, the condition is sufficient for complete dephosphorylation. If you use ten times or more the amount of PAP shown in the table 2, we recommend phenol extraction to inactivate PAP completely instead of heat inactivation (see *4 and *5).

*4 Non-diluted PAP solution should be added not exceeding 10% in a volume of the final reaction buffer. Glycerol in non-diluted PAP solution and high concentration of PAP protein inhibit heat inactivation of PAP. If the reaction mixture contains non-diluted PAP solution up to 20% of its volume, the activity of about 1/7,000 still remains. When you use non-diluted PAP solution over 10% volume of reaction mixture, we recommend phenol extraction for complete inactivation of PAP (see *5).

*5 Heat inactivation

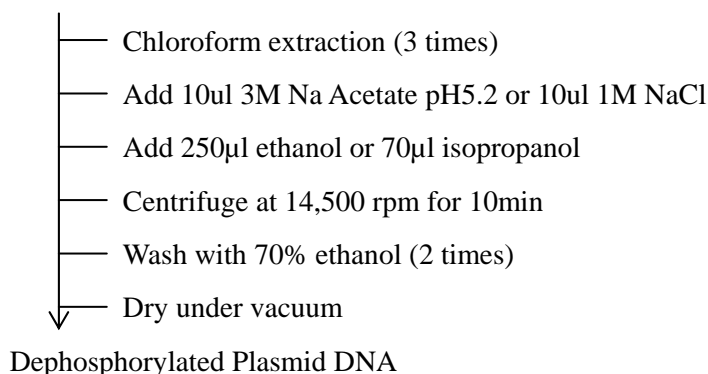
At least 10 µl of reaction mixture is required in 0.5ml tube for heat inactivation. Before heat inactivation, you should spin down solution in the tube. After heat inactivation, the white precipitate of inactivated PAP protein is observed. It is not necessary to be removed, because the precipitate does not affect the next ligation reaction.

Although almost of all PAP are inactivated by incubation at 95 °C for 5min after dephosphorylation, the activity of 1/100,000 ~ 1/400,000 remains. It is negligible level in the next ligation reaction, because the residual PAP activity can only dephosphorylate insert DNA (foreign) less than 1/10,000 of the initial vector plasmid DNA in the molar amount, if you follow the above protocol.

If you use more amount of enzyme than that of above condition (table2) or use over 10% volume of final reaction mixture, we recommend phenol extraction to inactivate PAP completely instead of heat inactivation as follows;

Alkaline phosphatase reaction product
└─ Phenol/chloroform (1:1) extraction

PRODUCT INFORMATION



*6 It is possible that alkaline phosphatase reaction mixture is directly added to ligation reaction mixture up to 30% for cohesive end ligation, up to 10% for blunt end ligation of the final reaction volume without interference.

The commercially available ligation kit such as DNA Ligation Kit ver.2 (TaKaRa), Ligation High (TOYOBO CO., LTD), Quick Ligation™ Kit (New England Biolabs. Inc) and LigaFast™ Rapid DNA Ligation Kit (Promega) can be effectively used in instead of this ligation protocol. During you use these kits, the orange precipitate derived from reduced Ni is observed. It is not necessary to remove the precipitate because it does not affect the ligation and the next transformation process.

*7 Chill the reaction mixture of insert DNA and vector DNA to 0 before ligation procedure.

The molar ratio of vector DNA to insert DNA should be between 1:1 and 1:6. The final DNA concentration of vector DNA and insert DNA should be between 1ng/μl and 10ng/μl for an effective ligation.

*8 10 × Ligation buffer

660mM Tris-HCl (pH7.6), 66mM MgCl₂, 100mM DTT

*9 Amount of ligation mixture should be added in a volume not exceeding 10% of that of

PRODUCT INFORMATION

competent cells. If you carry out transformation of *E.coli* by electroporation, we recommend spin column purification of ligation mixture.

LigaFast™ and Quick Ligation™ are trademarks of Promega Corporation and New England Biolabs, Inc., respectively.

BioDynamics Laboratory Inc.