Certificate of Analysis & Product Manual

Electrophoresis Reagents, Polymerase Chain Reaction
Custom Primers and Probes; Fluorescent Probes, siRNA
Hybridization and Detection Reagents

Adaptors & Linkers

Cloning & Ligation

Catalog No.: 26-3100-XX and 26-3200-XX

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Material Supplied

Quantity	20 µg
Shipping Condition	Ambient
Storage	-20°C

Content	Catalog No.	Description	Sequence	MW	nmols/20 µg
❑	26-3200-02	BamH I linker non-phosphorylated	5′-CGGGATCCCG-3′ 3′-GCCCTAGGGC-5′	6,120	3.27
❑	26-3200-03	BamH I linker phosphorylated	5′-pCGGGATCCCG-3′ 3′--GCCCTAGGGCp-5′	6,280	3.27
❑	26-3200-04	Bgl II linker non-phosphorylated	5′-GAAGATCTTC-3′ 3′-CTTCTAGAAG-5′	6,116	3.27
❑	26-3200-05	Bgl II linker phosphorylated	5'-pGAAGATCTTC-3' 3′--CTTCTAGAAGp-5′	6,276	3.27
❑	26-3200-06	Cla I non-phosphorylated linker	5'-CCATCGATGG-3' 3′-GGTAGCTACC-5′	6,118	3.27
❑	26-3200-07	Cla I phosphorylated linker	5'-pCCATCGATGG-3' 3′--GGTAGCTACCp-5′	6,278	3.27
❑	26-3200-08	Eco RI non-phosphorylated linker	5'-CCGGAATTCCGG -3' 3′-GGCCTTAAGGCC-5′	7,354	2.71
❑	26-3200-09	Eco RI phosphorylated linker	5'-pCCGGAATTCCGG-3' 3′--GGCCTTAAGGCCp-5′	7,514	2.71
❑	26-3200-10	Hind III non-phosphorylated linker	5'-CCAAGCTTGG-3' 3′-GGTTCGAACC-5′	6,118	3.26
❑	26-3200-11	Hind III phosphorylated linker	5'-pCCAAGCTTGG-3' 3′--GGTTCGAACCp-5′	6,278	3.26
❑	26-3200-12	Nco I non-phosphorylated linker	5'-CATGCCATGGCATG-3' 3′-GTACGGTACCGTAC-5′	8,589	2.32
❑	26-3200-13	Nco I phosphorylated linker	5'-pCATGCCATGGCATG-3' 3′--GTACGGTACCGTACp-5′	8,749	2.32
❑	26-3200-14	Not I non-phosphorylated linker	5'-AGCGGCCGCT-3' 3′-TCGCCGGCGA-5′	6,120	3.26
❑	26-3200-15	Not I phosphorylated linker	5'-pAGCGGCCGCT-3' 3′--TCGCCGGCGAp-5′	6,280	3.26
❑	26-3200-16	Pst I non-phosphorylated linker	5'-GCTGCAGC-3' 3′-CGACGTCG-5′	4,883	4.09
❑	26-3200-17	Pst I phosphorylated linker	5'-pGCTGCAGC-3' 3′--CGACGTCGp-5′	5,043	4.09
❑	26-3200-18	Pvu I non-phosphorylated linker	5'-ATCGATCGAT -3' 3′-TAGCTAGCTA-5′	6,116	3.27
❑	26-3200-19	Pvu I phosphorylated linker	5'-pATCGATCGAT -3' 3′--TAGCTAGCTAp-5′	6,276	3.27
❑	26-3200-20	Sma I non-phosphorylated	5'-TCCCCCGGGGGA -3' 3′-AGGGGGCCCCCT-5′	7,356	2.71
❑	26-3200-21	Sma I phosphorylated linker	5'-pTCCCCCGGGGGA -3' 3′--AGGGGGCCCCCTp-5′	7,516	2.71

Content	Catalog No.	Description	Sequence	MW	nmols/20 μg
❑	26-3200-22	Xba I non-phosphorylated linker	5'-CTAGTCTAGACTAG -3' 3′-GATCAGATCTGATC-5′	8,587	2.32
❑	26-3200-23	Xba I phosphorylated linker	5'-pCTAGTCTAGACTAG-3' 3′--GATCAGATCTGATCp-5′	8,747	2.32
❑	26-3200-24	Xho I non-phosphorylated linker	5'-CCGCTCGAGCGG-3' 3′-GGCGAGCTCGCC-5′	7,356	2.71
❑	26-3200-25	Xho I phosphorylated linker	5'-pCCGCTCGAGCGG-3' 3′-GGCGAGCTCGCCp-5′	7,516	2.71
❑	26-3200-26	Nhe I non-phosphorylated linker	5'-GGCTAGCC-3' 3′-CCGATCGG-5′	4,883	4.09
❑	26-3200-27	Nhe I phosphorylated linker	5'-pGGCTAGCC-3' 3′--CCGATCGGp-5′	5,043	4.09
❑	26-3200-28	Apa I non-phosphorylated linker	5'-GGGGCCCC -3' 3′-CCCCGGGG-5′	4,885	4.09
❑	26-3200-29	Apa I phosphorylated linker	5'-pGGGGCCCC-3' 3′--CCCCGGGGp-5′	5,045	4.09
❑	26-3200-30	Eag I non-phosphorylated linker	5'-CCGGCCGG-3' 3′-GGCCGGCC-5′	4,885	4.09
❑	26-3200-31	Eag I phosphorylated linker	5'-pCCGGCCGG-3' 3′--GGCCGGCCp-5′	5,045	4.09
❑	26-3200-32	Pac I non-phosphorylated linker	5'-GTTAATTAAC-3' 3′-CAATTAATTG-5′	6,114	3.27
❑	26-3200-33	Pac I phosphorylated linker	5'-pGTTAATTAAC-3' 3′--CAATTAATTGp-5′	6,274	3.27
❑	26-3200-34	Kpn I non-phosphorylated linker	5'-GGGTACCC-3' 3′-CCCATGGG-5′	4,883	4.09
❑	26-3200-35	Kpn I phosphorylated linker	5'-pGGGTACCC-3' 3′--CCCATGGGp-5′	5,043	4.09
❑	26-3200-36	Mlu I non-phosphorylated linker	5'-CGACGCGTCG -3' 3′-GCTGCGCAGC-5′	6,120	3.26
❑	26-3200-37	Mlu I phosphorylated linker	5'-pCGACGCGTCG-3' 3′--GCTGCGCAGCp-5′	6,280	3.26
❑	26-3200-38	Spe I non-phosphorylated linker	5'-GGACTAGTCC-3' 3′-CCTGATCAGG-5′	6,118	3.26
❑	26-3200-39	Spe I phosphorylated linker	5'-pGGACTAGTCC-3' 3′--CCTGATCAGGp-5′	6,278	3.26

Material Supplied

One tube containing oligo linker annealed as a double strand. The product is supplied as a lyophilized powder. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Reconstitution

Reconstitute oligo linker in sterile water pH 7.0 or TE pH 7.0 preferably at 100 µM concentration. Gene Link provides the total nmol supplied (see above). For example if the total quantity is 3 nmols then dissolve in 30 µL. Traditionally molecular biology labs reconstitute at 1 µg/ µL but this becomes tedious to convert back to pmol ends for ligation purposes.

Linkers are short oligos that are supplied in an annealed form. Due to the short size the Tm is low and thus even room temperature storage should be avoided. Always keep them on ice when in use.

Certificate of Analysis & Product Specifications

Linker sequences are chemically synthesized as two separate single strands and then annealed at equimolar quantity and then lyophilized. They have been validated for ligation and restriction enzyme digestion.

Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

All linkers pass the above specifications and are certified to be nuclease free. Appropriate nuclease free handling, dispensing and storage conditions required.

Lot Number

Manufacturing lot number is stated on the label of product and accompanying packing slip.

Product Description & Application

Gene Link supplied linkers are short synthetic oligonucleotide pre-annealed duplexes. These can be ligated to the DNA template of interest by blunt end ligation. These have the specified internal restriction endonuclease site.

Linkers are used for various cloning strategies to introduce restriction sites in the DNA after ligation. Linkers are short synthetic palindromic sequences that self-anneal to form blunt ended double stranded fragments. Linkers are supplied as phosphorylated and non-phosphorylated forms.

Adaptors Vs Linkers

The choice of use of either a linker or an adaptor is based on the application and the ends of the DNA of interest that needs to be altered as it does not have the desired restriction site at the end nor has that site internally that will digest the DNA to several fragments. Preference should be given to ligation using cohesive ends as these are ligated more efficiently than blunt ends, i.e. use adaptors over linkers.

An easy example is when the DNA of interest is required to be cloned in a vector using Eco RI site of the vector. This requires the insert DNA to have the Eco RI site as the ends to generate cohesive overhangs. In this example Adaptors are designed such that the sticky cohesive over hang contain half site of a specific DNA sequence corresponding to the specific restriction enzyme.

Linkers are synthesized as single stranded oligos with the restriction site in the middle and designed to have a palindrome sequence. These single strands self-ligate to form a double strand. See figure below.

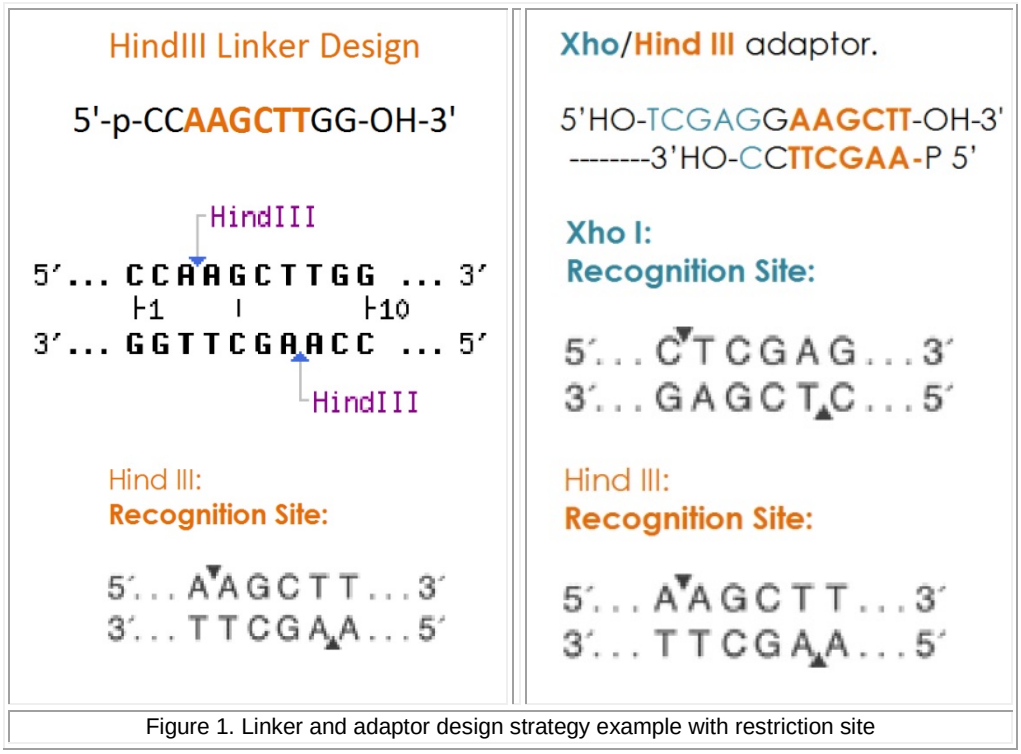

Figure 1. Linker and adaptor design strategy example with restriction site

Ligation

1. Follow ligase provider protocol for ligation.
2. Visit Gene Link web site for Ligation Calculator to determine ratio of insert:vector at the following link http://www.genelink.com/tools/gl-lc.asp

3. Conditions for adaptor ligation is the same as for insertion of DNA fragments into a plasmid vector. The recommended molar ratio of phosphorylated adaptor:dephosphorylated vector is 10-100:1. When using phosphorylated vector, the adaptor:vector molar ratio should be >100:1.

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Related Products Ordering Information

DNA & RNA Reconstitution Solutions

Product	Catalog No.	Unit Size
DNA & RNA Reconstitution Solutions Pack (contains 50 mL each of DEPC Treated Water [40-3000-05], Nuclease Free Water (DEPC Free) [40-3001-05], TE pH 7.0 [40-5011-05] and RNA Reconstitution Solution[40-5014-05)	40-3000-00	1 Pack
RNA Reconstitution Solution (1 mM Sodium Citrate pH 6.4) 10 X 1.6 mL	40-5014-16	10 X 1.6 mL
RNA Reconstitution Solution (1 mM Sodium Citrate pH 6.4); 50 mL	40-5014-05	50 mL
TE Buffer 1X solution pH 7.0; 50 mL	40-5011-05	50 mL
TE Buffer 1X solution pH 7.5; 50 mL	40-5012-05	50 mL
TE Buffer 1X solution pH 8.0; 50 mL	40-5013-05	50 mL
Nuclease Free Water (DEPC Free) 10 X 1.6 mL	40-3001-16	10 X 1.6 mL
Nuclease Free Water (DEPC Free) 50 mL	40-3001-05	50 mL
Nuclease Free Water (DEPC Free) 500 mL	40-3001-50	500 mL
Nuclease Free Water (DEPC Free); 1L	40-3001-01	1 L
DEPC Treated Water; 10 X 1.6 mL	40-3000-16	10 X 1.6 mL
DEPC Treated Water; 50 mL	40-3000-05	50 mL
DEPC Treated Water; 500 mL	40-3000-50	500 mL
DEPC Treated Water; 1L	40-3000-01	1 L

Related Products Ordering Information

DNA & RNA Precipitation Solutions

Product	Catalog No.	Unit Size
DNA & RNA Precipitation Solutions Pack (contains the following; Glycogen Solution 10 mg/mL; 1 mL [40-5112-01]; Linear Acrylamide Solution 5mg/mL; 1 mL [40-5113-01] LiCl RNA Precipitation Solution [40-5131-05]; Sodium Acetate DNA & RNA Precipitation Solution [40-5132-05]; Sodium Chloride DNA & RNA Precipitation [40-5134-05] and Ammonium Acetate 7.5M DNA & RNA Precipitation Solution [40-4135-05])	40-5130-00	1 Pack
Glycogen Solution 10 mg/mL; 1 mL	40-5112-01	1 mL
Linear Acrylamide Solution (Linear polyacrylamide, LPA; 5mg/mL); 1 mL	40-5113-01	1 mL
LiCl RNA Precipitation Solution (7.5M LiCl, 50 mM EDTA pH 8.0); 50 mL	40-5131-05	50 mL
Sodium Acetate DNA & RNA Precipitation Solution (3M Sodium Acetate pH 5.5); 50 mL	40-5132-05	50 mL
Potassium Acetate DNA & RNA Precipitation Solution (3M Potassium Acetate pH 5.5); 50 mL	40-5133-05	50 mL
Sodium Chloride DNA & RNA Precipitation (5M Sodium Chloride); 50 mL	40-5134-05	50 mL
Ammonium Acetate DNA & RNA Precipitation Solution (7.5M Ammonium Acetate); 50 mL	40-5135-05	50 mL
Ammonium Acetate DNA & RNA Precipitation Solution (5M Ammonium Acetate); 50 mL	40-5136-05	50 mL

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Related Products Ordering Information

PCR Additives & Reagents

Product	Catalog No.	Unit Size
Taq DNA Polymerase 300 units; 5 µ/µL; 60 µL	40-5200-30	300 units
PCR Buffer Standard (10 X); 1.6 mL	40-3060-16	1.6 mL
PCR Buffer Mg Free (10 X); 1.6 mL	40-3061-16	1.6 mL
Taq Polymerase Dilution Buffer; 1 mL	40-3070-10	1 mL
dNTP 2mM (10X); 1.1 mL	40-3021-11	1.1 mL
$MgCl_2$; 25 mM; 1.6 mL	40-3022-16	1.6 mL
Omni-Marker™ Universal Unlabeled; 1 mL	40-3005-10	1 mL
Primer and Template Mix; 500 bp; 40 reactions	40-2026-60PT	100 µL
Nuclease Free Water, 10 X 1.6 mL	40-3001-16	10 X 1.6 mL
DMSO, 1 mL	40-3031-10	1 mL
TMAC (Tetramethyl ammonium chloride) 100 mM; 1 mL	40-3053-10	1 mL
KCl 300 mM; 1 mL	40-3059-10	1 mL
Betaine 5M; 1 mL	40-3032-10	1 mL

Omni-Marker™; Molecular Weight Size Standards for Gel Electrophoresis

Product	Catalog No.	Unit Size
Omni- Marker™ DNA 1 kb mw Universal unlabeled; 500 µL	40-3005-05	500 µL
Omni-Marker™ DNA 1 kb mw Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni-Marker™ DNA 100 bp mw Low unlabeled; 500 µL	40-3006-05	500 µL
Omni- Marker™ DNA 100 bp mw Low unlabeled; 1 mL	40-3006-10	1 mL

Loading Buffers; DNA non-denaturing and denaturing buffers

Product	Catalog No.	Unit Size
Loading Buffer 5X BPB/XC non-denaturing; 1 mL	40-3002-10	1 mL
Loading Buffer 5X BPB/XC non-denaturing; 15 mL	40-3002-15	15 mL
Loading Buffer 5X Orange G/XC non-denaturing; 1 mL	40-3004-10	1 mL
Loading Buffer 5X Orange G/XC non-denaturing; 15 mL	40-3004-15	15 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL	40-5027-15	15 mL

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