BIOLINE	**Certificate of Analysis**	**COA No: CA BB-0020**
		Version: 02

ER Buffer, 5x	Storage Conditions:	-20°C
	Lot number:	ERB-717108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
Functional	Using ligation and subsequent amplification the efficiency of ER Buffer (test) is measured against a positive control representing a 100% efficiency. The difference in efficiency is measured by the ΔCt between the second dilution of the positive control and the test which is constant and equal to 10 ± 1.5.	ΔCt = 8.98
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with a reference sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441

BIOLINE	**Certificate of Analysis**	**COA No: CA BEM-0029**
		Version: 02

ER Enzyme Mix	Storage Conditions:	-20°C
	Lot number:	ERM-717108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
Functional	Using ligation and subsequent amplification the efficiency of ER Enzyme Mix (test) is measured against a positive control representing a 100% efficiency. The difference in efficiency is measured by the ΔCt between the second dilution of the positive control and the test which is constant and equal to 10 ± 1.5.	ΔCt = 8.98
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with a reference sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441

BIOLINE	**Certificate of Analysis**	**COA No: CA BB-0051**
		Version: 02

Ligase Buffer, 5x	Storage Conditions:	-20°C
	Lot number:	LGB-717108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
Functional	Using ligation and subsequent amplification the efficiency of the ligase buffer is measured against a positive control representing 100% efficiency. The difference in efficiency is measured by the ΔCt between the test reaction and the positive control which is constant and equal to 10.7 ±0.3.	ΔCt = 10.97
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with a reference sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441

enzymatics®
a QIAGEN company

Certificate of Analysis *L6030 Rev F*

Product Information	
T4 DNA Ligase	
Sub-Part Number	L6030-600,000
Concentration	600,000 U/mL
Unit Size	11,400,000 U
Sub-Lot Number	40060817

Product Lot Information	
Master Part Number	**L6030**
Master Lot Number	**40**
Expiration Date	**09/2018**
Recommended Storage	-25°C to -15°C

Product Specifications						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**
Units Tested	**n/a**	**n/a**	**6,000**	**6,000**	**6,000**	**6,000**
Specification	>99%	300,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies
Actual	>99%	300,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Lot Release Approval: _____
Director Quality Control

Product Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and a 3' hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Source of Protein: A recombinant *E. coli* strain carrying the cloned T4 DNA Ligase gene.

Unit Definition: 1 unit is defined as the amount of DNA Ligase required to join 50% of 100 ng of DNA fragments with cohesive termini in 50 µl 1X DNA Ligase Buffer following a 30 minute incubation at 23°C

Molecular weight: 55,292 Daltons

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X DNA Ligase Reaction Buffer and added to 20 µL reactions containing double stranded DNA fragments and 1X DNA Ligase Reaction Buffer. Reactions are incubated for 30 minutes at 23°C, stopped, and analyzed on a 1% agarose gel stained with ethidium bromide.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

enzymatics® a QIAGEN company

Certificate of Analysis *L6030 Rev F*

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in: 10mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol pH 7.5 @ 25ºC.

Notes:
One Enzymatics T4 DNA Ligase cohesive end unit is equivalent to approximately 3 cohesive end units as measured with a Lambda-Hind III DNA fragment substrate in 1X T4 DNA Ligase reaction buffer. One Weiss Unit is approximately equivalent to 22 Enzymatics cohesive end units.
T4 DNA Ligase is ATP dependent. It is recommended that the reaction buffer be discarded after one year of storage at -20°C and replaced with fresh buffer to ensure maximum performance.
Single-insert ligations are optimal when targeting an insert:vector ratio between 2 and 6. A ratio above 6:1 will promote the insertion of multiple fragments, while dropping below 2:1 will reduce ligation efficiency. For problematic ligations or if the DNA concentration is unknown, it may be necessary to vary ratios and run multiple ligations
Enzymatics 10X T4 DNA Ligase Buffer does not contain PEG and is compatible with standard ligation protocols which do not specify the use of a rapid/fast/quick format buffer.

References: 1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.), The Enzymes, 5, pp. 3. San Diego: Academic Press.

BIOLINE	**Certificate of Analysis**	**COA No: CA_BS-0020**
		Version: 02

DEPC Water	Storage Conditions:	-20°C
	Lot number:	DWT-717108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with control sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase I.	Passed
RNase contamination	Quantitative PCR analysis with high and low RNase standards. Test sample must show less RNase activity than the limit of detection 9.7x10^{-3} ng/µL RNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441

BIOLINE	**Certificate of Analysis**	**COA No: CA BB-0057**
		Version: 02

PCR Buffer, 10x	Storage Conditions:	-20°C
	Lot number:	717108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
Functional	Fragment size of 1200bp is amplified from a specific dilution series of template DNA under defined conditions. The reactions are analysed using agarose gel electrophoresis (ethidium stained). A 1200bp band should be observed from dilution 1 to 9.	Passed
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with a reference sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441

BIOLINE	**Certificate of Analysis**	**COA No: CA BEM-0004**
		Version: 02

ACCUZYME DNA Polymerase	Storage Conditions:	-20°C
	Lot number:	AC-617108
For Research Use Only	Expiry date:	September 2019

Quality Control Parameters

Analysis	Specification	Result
Functional	Fragment of size 3Kb is amplified with a dilution series Lambda DNA, using standard conditions and 30 cycles. Fragment of size 5Kb is amplified with a dilution series of Lambda DNA, using standard conditions and 30 cycles. Single distinct bands were observed with agarose gel electrophoresis (ethidium stained). Quantitative PCR analysis amplifying 1 gene from a dilution series of enzyme under standard conditions. Cq and melting profiles must be consistent for the test and reference sample with 0.5+/- Cq variance.	Passed
DNA contamination	Quantitative PCR analysis with no template. Presence of *E. coli* and mouse genomic DNA checked. Test sample must amplify in line with a reference sample.	Passed
DNase contamination	Incubation of a 1Kb double stranded DNA fragment. Incubation for 4 hours at 37°C with dilution series of DNase I. Analysed by agarose gel electrophoresis. Test sample must show less degradation than the limit of detection 2.5 x 10^{-3} U DNase.	Passed

Authorised by Christopher Weatherall

United Kingdom
Headquarters UK
info.uk@bioline.com
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

USA
info.us@bioline.com
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Germany
info.de@bioline.com
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

France
Info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Australia
info.aust@bioline.com
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Singapore
Info.sg@bioline.com
Tel: +65 6774 7196
Fax +65 6774 6441