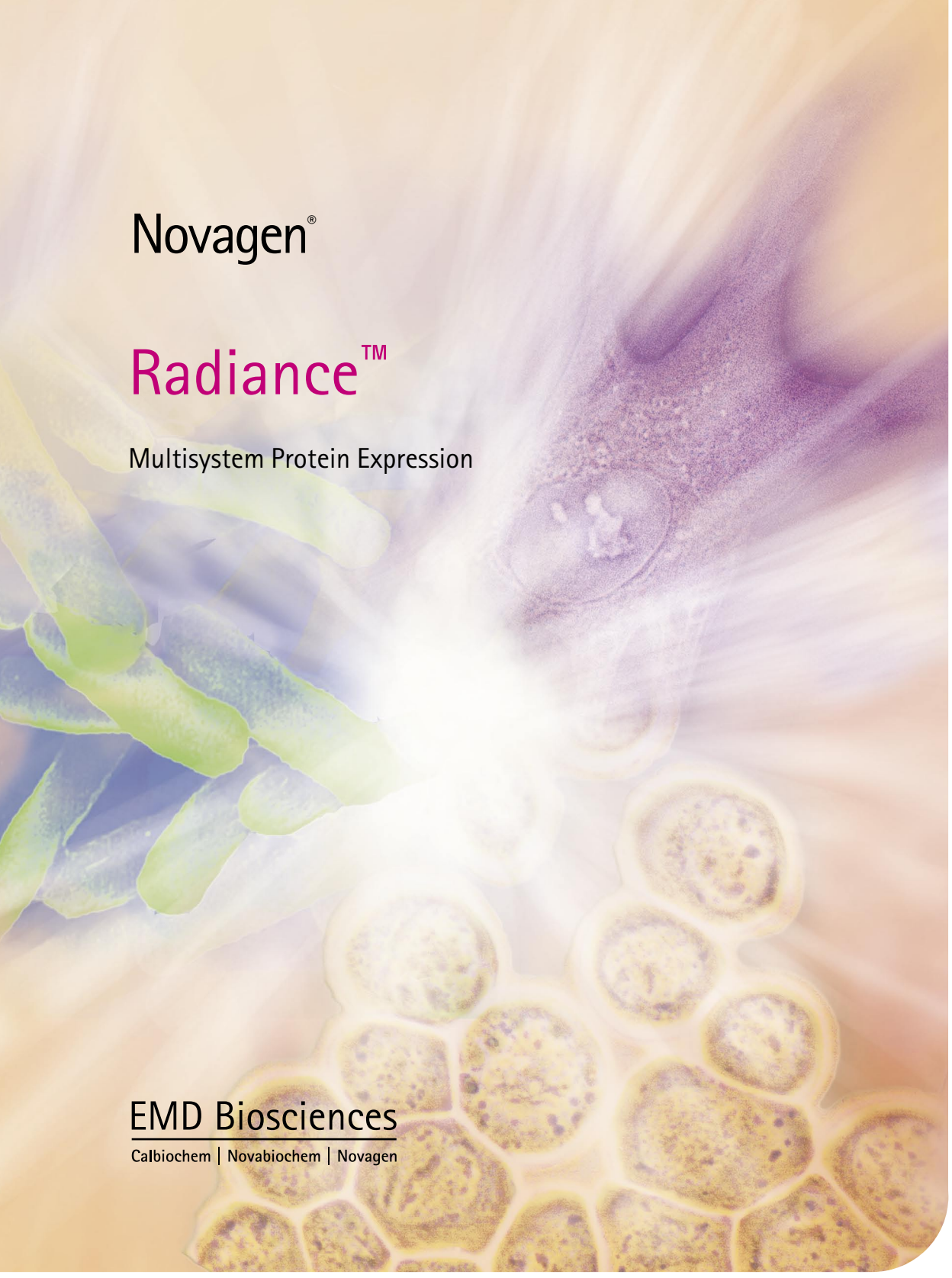


Novagen®

Radiance™

Multisystem Protein Expression

EMD Biosciences

Calbiochem | Novabiochem | Novagen

EMD

Why Radiance?

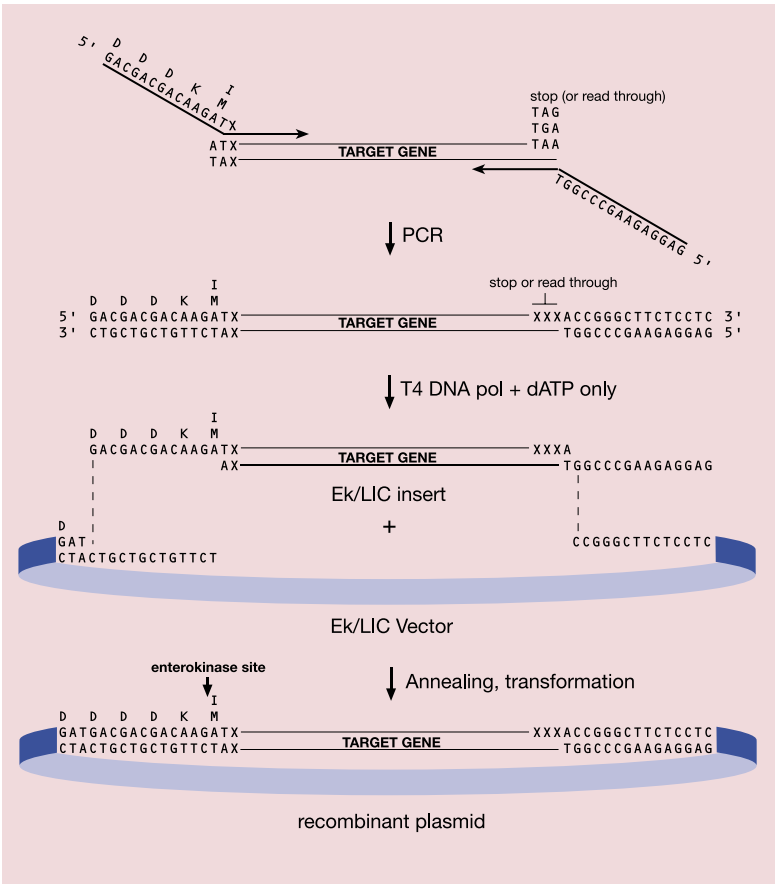
The Radiance™ Cloning and Expression System provides ready-to-use expression vectors for efficient, directional cloning of PCR products and their subsequent expression in bacterial, insect, and mammalian cells. The system offers an alternative to recombinase-mediated cloning with the unique ability to remove all amino-terminal vector-encoded sequences from the target protein after purification.

- One insert for ligation-independent cloning into many available vectors
- Multiple expression systems: bacterial, insect, and mammalian
- No extra vector-encoded amino acids
- Coexpression of multiple proteins in one cell

One Insert for Ligation-independent Cloning

Efficient, directional cloning of PCR products and their subsequent expression in bacterial, insect, and mammalian cells

By design, the prepared insert is compatible with every Novagen-prepared Ek/LIC vector and enables insertion into multiple vectors. Clone directly into any of the vectors in the Radiance Cloning and Expression System, which encompasses the entire Novagen offering of enterokinase (Ek) ligation-independent cloning (LIC) vectors. The LIC method enables high-efficiency directional cloning without restriction enzyme digestion or ligation reactions (1, 2). Novagen Ek/LIC Vectors are prepared to have very specific, noncomplementary 13- and 14-nucleotide single-stranded overhangs. PCR products with overhangs complementary only to the vector are easily created by building 5′-extensions into the primers followed by treatment with LIC-qualified T4 DNA Polymerase in the presence of dATP. After brief annealing with the vector and transformation, virtually all of the resulting colonies contain the desired recombinant.



Multiple Expression Systems: Bacterial, Insect, and Mammalian

Bacterial

- Powerful T7*lac* promoter vectors with popular tags for enhanced solubility and purification
- Compatible vectors for coexpression

Insect

- Vectors for rapid, high-level expression in transfected cells without the need for making a recombinant baculovirus
- Traditional baculovirus transfer plasmids

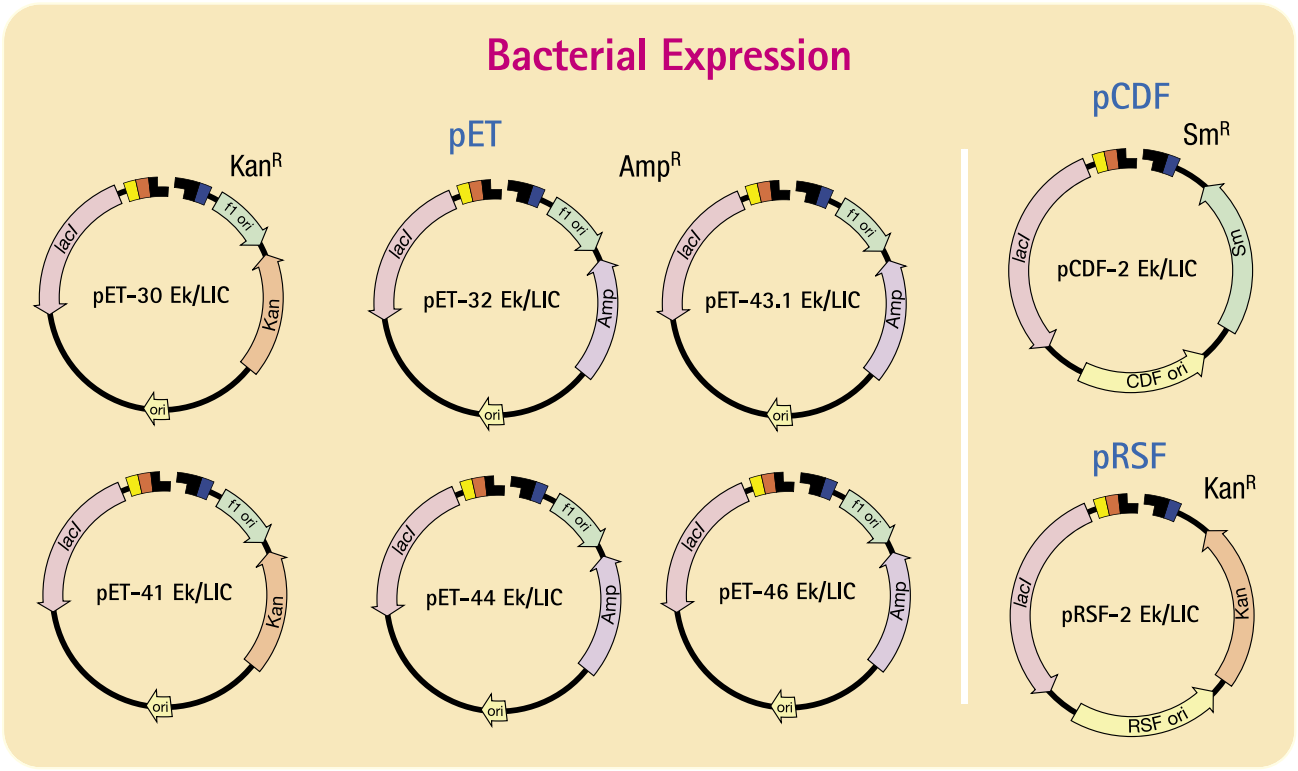
Mammalian/Multisystem

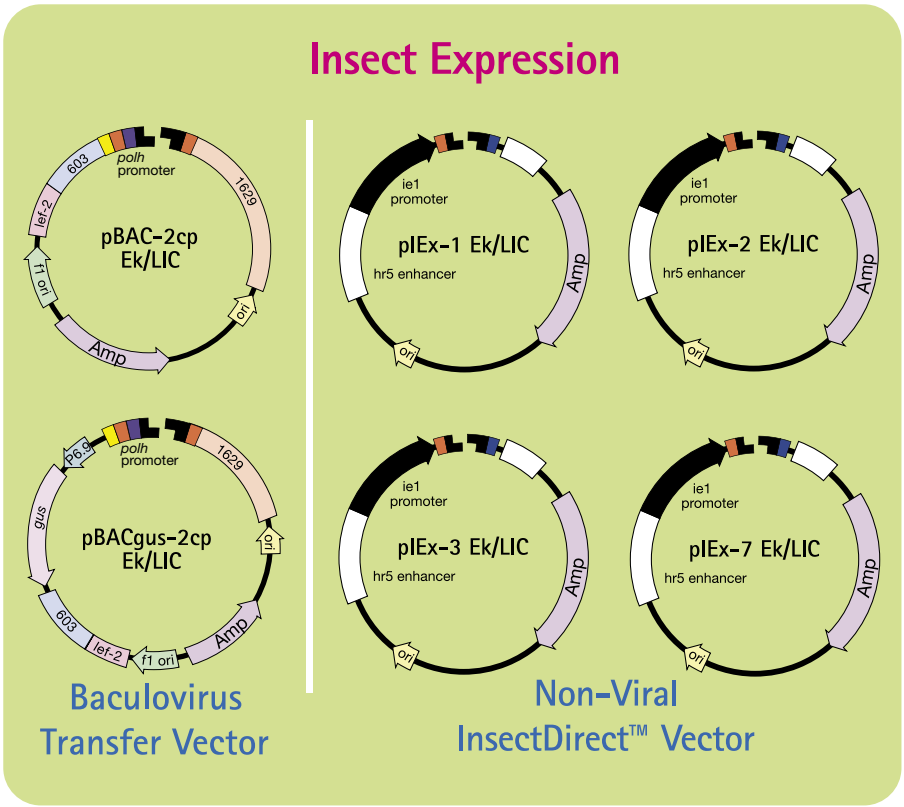
- Vector for high-level expression in bacterial, insect, and mammalian systems

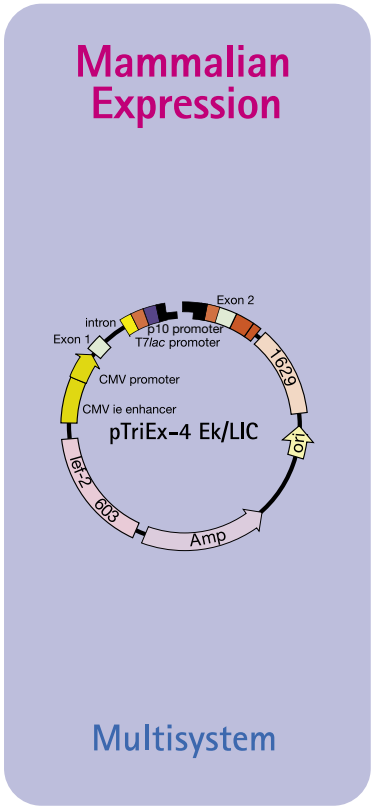
No Extra Vector-encoded Amino Acids

Amino acids that are not part of the natural protein sequence may affect activity interactions and structure. An important feature of the LIC method and the Novagen Ek/LIC vector design is that all amino-terminal vector-encoded sequences can be removed with Recombinant Enterokinase digestion following purification.

Radiance™ Cloning and Expression System







Technical Support
Phone 800 207 0144
E-mail novagen@emdbiosciences.com

Coexpression of multiple proteins in *E. coli*

Coexpress Up to Six Proteins in One Cell

Coexpression of multiple open reading frames (ORFs) in *E. coli* can enhance yield, solubility, and activity of proteins that either are part of a multi-protein complex or benefit from coexpression with chaperones. Coexpression also can facilitate the analysis of multi-subunit complexes and biochemical pathways and the characterization of protein-protein interactions, among other applications (3).

Expression of multiple ORFs can be achieved two ways: transform cells with two or more plasmids having compatible replicons and different drug resistance genes or express two ORFs from a single construct using the LIC Duet™ Adaptors described on the facing page. The pRSF-2 Ek/LIC and pCDF-2 Ek/LIC vectors contain ColE1-compatible replicons and allow coexpression of up to three proteins when present in the same cell together with an ampicillin-resistant pET vector construct. Other T7 promoter-based vectors that are compatible for coexpression with Radiance™ vectors include the Duet series and pETcoco™ vectors.

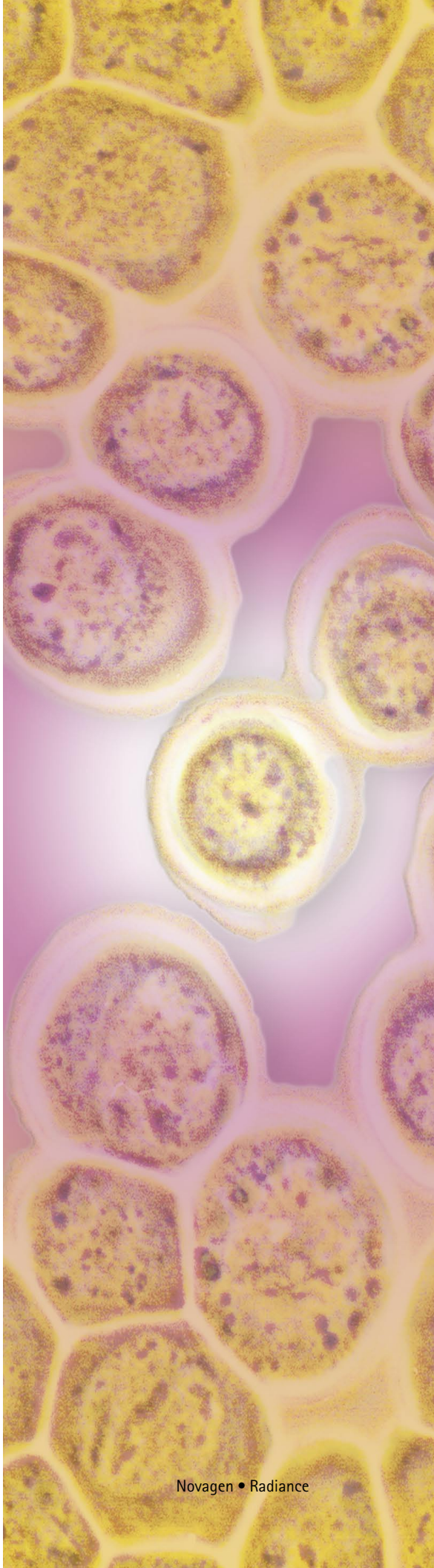
Vector and host strain compatibilities for coexpression of four to six target proteins

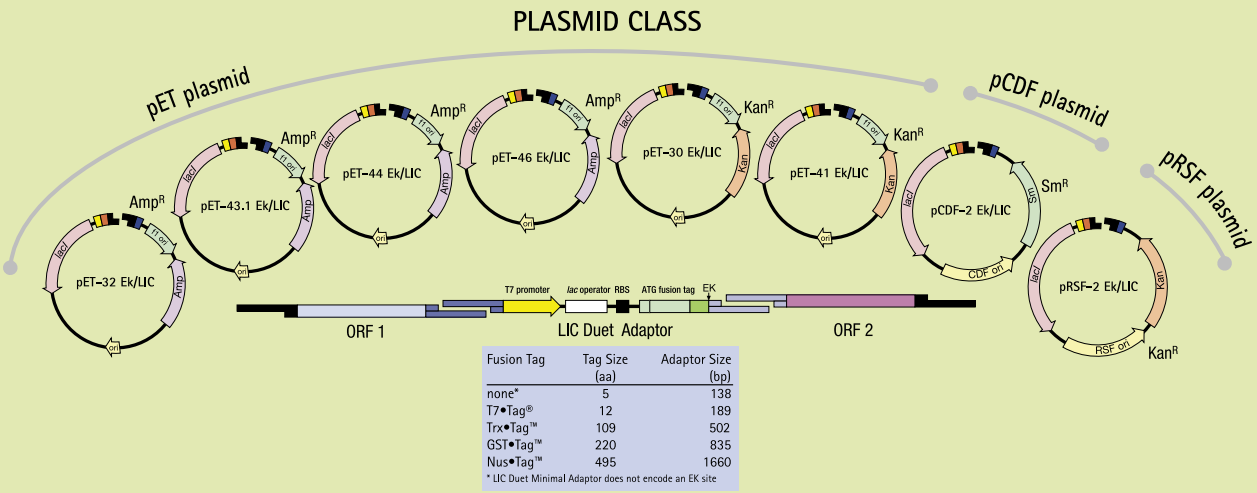
Compatible Vector Combinations			Number of Possible Target Proteins*	Compatible Host Strain Group†
Vector 1	Vector 2	Vector 3		
pET Ek/LIC (Amp^R)	pRSF-2 Ek/LIC (Kan^R)	pCDF-2 Ek/LIC (Sm^R)	6	A
pET (Amp^R)	pRSF-2 Ek/LIC (Kan^R)	pCDF-2 Ek/LIC (Sm^R)	5	A
pET Ek/LIC (Amp^R)	pRSF-2 Ek/LIC (Kan^R)	none	4	A
pET Ek/LIC (Amp^R)	pCDF-2 Ek/LIC (Sm^R)	none	4	B
pRSF-2 Ek/LIC (Kan^R)	pCDF-2 Ek/LIC (Sm^R)	none	4	A

Host strain groups

Group A		Group B	
B834(DE3)	Rosetta 2(DE3)	B834(DE3)	Rosetta(DE3)
B834(DE3)pLysS	Rosetta 2(DE3)pLysS	B834(DE3)pLysS	Rosetta(DE3)pLysS
BL21(DE3)	Rosetta-gami™ 2(DE3)††	BL21(DE3)	Rosetta 2(DE3)
BL21(DE3)pLysS	Rosetta-gami 2(DE3)pLysS††	BL21(DE3)pLysS	Rosetta 2(DE3)pLysS
BLR(DE3)	RosettaBlue™(DE3)	BLR(DE3)	RosettaBlue(DE3)
BLR(DE3)pLysS	RosettaBlue(DE3)pLysS	BLR(DE3)pLysS	RosettaBlue(DE3)pLysS
HMS174(DE3)	Tuner™(DE3)	HMS174(DE3)	Rosetta-gami™(DE3)††
HMS174(DE3)pLysS	Tuner(DE3)pLysS	HMS174(DE3)pLysS	Rosetta-gami(DE3)pLysS††
NovaBlue(DE3)		NovaBlue(DE3)	Rosetta-gami 2(DE3)††
Rosetta™(DE3)		Origami™(DE3)††	Rosetta-gami 2(DE3)pLysS††
Rosetta(DE3)pLysS		Origami(DE3)pLysS††	Rosetta-gami B(DE3)
		Origami B(DE3)	Rosetta-gami B(DE3)pLysS
		Origami B(DE3)pLysS	Tuner(DE3)
			Tuner(DE3)pLysS

Resistance markers: Amp^R, ampicillin/carbenicillin; Kan^R, kanamycin; Sm^R, streptomycin/spectinomycin
*Assumes two target genes are cloned for each Ek/LIC vector using the LIC Duet Adaptor method
†Because drug resistance plays a critical role in the success of coexpression applications, host strains, which may also confer their own resistance, must be selected carefully.
††These strains carry the *rpsL* mutation that confers resistance to streptomycin; therefore, spectinomycin must be used for selection of pCDFDuet™ recombinants.





Fusion Tag	Tag Size (aa)	Adaptor Size (bp)
none*	5	138
T7•Tag®	12	189
Trx•Tag™	109	502
GST•Tag™	220	835
Nus•Tag™	495	1660

* LIC Duet Minimal Adaptor does not encode an EK site

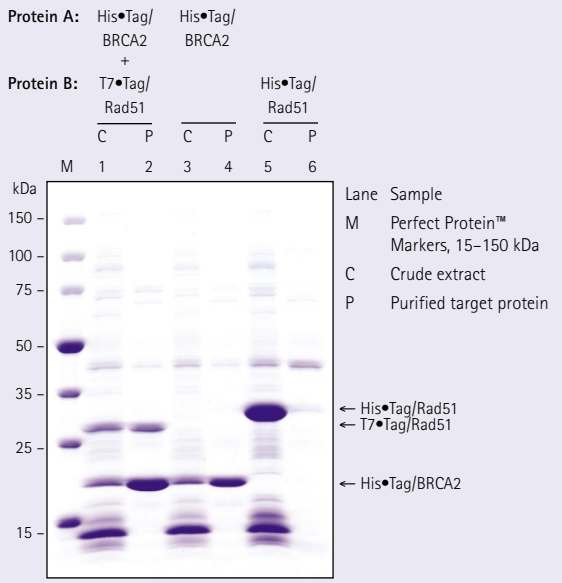
Compatible Ek/LIC vectors for coexpression in bacteria

LIC Duet Adaptors

LIC Duet™ Adaptors can convert any Novagen bacterial Ek/LIC-prepared plasmid into a coexpression vector by allowing two ORFs to be cloned simultaneously and subsequently coexpressed in *E. coli*.

Four adaptors encode fusion tags which aid in purification and/or may enhance solubility of the target proteins. An enterokinase cleavage site follows each adaptor-encoded tag, allowing complete tag removal if desired. The fusion tags for glutathione S-transferase (GST•Tag), NusA (Nus•Tag), T7 (T7•Tag), and thioredoxin (Trx•Tag) are useful for fusion protein detection. The GST•Tag and T7•Tag versions of the adaptors facilitate purification; the GST, Nus, and Trx versions may enhance solubility of their fusion partner. A fifth adaptor, the LIC Duet Minimal Adaptor, is designed for expression of a target protein with a minimal fusion. This "mini" adaptor does not have an enterokinase cleavage site. The LIC Duet Adaptors:

- Allow coexpression of two proteins from one vector, or up to six proteins using an appropriate combination of up to three Ek/LIC vectors.
- Encode a T7*lac* promoter/operator, a strong ribosome binding site (RBS), and an ATG start codon.
- Anneal directionally to two ORFs, thereby creating an ORF-1/adaptor/ORF-2 annealed insert, which can then anneal to the overhangs of the Ek/LIC vectors.
- Are interchangeable: the five adaptors possess a common overhang upstream of the T7*lac* promoter and a different, yet common, overhang downstream of the ATG start codon.



His•Tag® fusions of BRCA2 and Rad51 were expressed alone in pET-30 Ek/LIC. The BRCA2 and Rad51 proteins were also coexpressed in pET-30 Ek/LIC vector using the LIC Duet™ T7•Tag® Ek Adaptor. The recombinant plasmids were transformed into Rosetta™(DE3) competent cells, grown in LB broth, and induced with IPTG at 26°C for 4 h. Cells were harvested by centrifugation and lysed with BugBuster® Protein Extraction Reagent, rLysozyme™ Solution, and Benzonase® Nuclease. Equal volumes were purified by Ni-NTA His•Bind® chromatography under native conditions. Samples representing equal cell mass were analyzed by SDS-PAGE (4–20% gradient) and stained with Coomassie blue.

REFERENCES:

1. Aslandis, C. and de Jong, P. J. (1990) *Nucleic Acids Res.* **18**, 6069-6074.
2. Haun, R. S., Servanti, I. M., and Moss, J. (1992) *BioTechniques* **13**, 515–518.
3. Novy, R., Yaeger, K., Held, D., and Mierendorf, R. (2002) *inNovations* **15**, 2–6.

Product	Size	Cat. No.	Price
LIC Duet™ Minimal Adaptor	20 rxn	71362-3	$169
LIC Duet T7•Tag® Ek Adaptor	20 rxn	71321-3	$169
LIC Duet Trx•Tag™ Ek Adaptor	20 rxn	71322-3	$169
LIC Duet GST•Tag™ Ek Adaptor	20 rxn	71323-3	$169
LIC Duet Nus•Tag™ Ek Adaptor	20 rxn	71324-3	$169

Components:

- 20 µl LIC Duet Ek or Minimal Adaptor
- 4 µl LIC Duet Control Insert 1
- 8 µl LIC Duet Control Insert 2

Radiance™ Buying Guide

LIC Vector Kit*	Expression System			N-terminal Tag					C-terminal Tag (optional)			Other Features						Cat. No.	Price
	Bacterial	Insect	Mammalian	GST•Tag™	His•Tag®	Nus•Tag™	S•Tag™	Trx•Tag™	His•Tag	HSV•Tag™	S•Tag	Cytoplasmic expression with enhanced solubility	Enhanced purification with His•Mag™ Agarose Beads	ColE1-compatible replicon for coexpression	GUS marker for baculovirus recombinants	Signal sequence for insect cell secretion of target protein	Thrombin (Tb) and Enterokinase (Ek) Protease cleavage sites		
pET-30 Ek/LIC[1]	×				×		×		×								Tb/Ek	69077-3	$368
pET-32 Ek/LIC[1]	×				×		×	×	×			×					Tb/Ek	69076-3	$368
pET-41 Ek/LIC[1]	×			×	×		×		×			×					Tb/Ek	71071-3	$368
pET-43.1 Ek/LIC[1]	×				×	×	×		×	×		×					Tb/Ek	71072-3	$368
pET-44 Ek/LIC[1]	×				×†	×	×		×	×		×	×				Tb/Ek	71144-3	$368
pET-46 Ek/LIC[1]	×				×†						×						Ek	71335-3	$368
pCDF-2 Ek/LIC[1]	×				×†						×			×			Ek	71337-3	$368
pRSF-2 Ek/LIC[1]	×				×†						×			×			Ek	71364-3	$368
pBAC™-2cp Ek/LIC[2]		×			×		×		×								Tb/Ek	70021-3	$343
pBACgus-2cp Ek/LIC[3]		×			×		×		×						×		Tb/Ek	70051-3	$343
pIEx™-1 Ek/LIC[3]		×			×		×			×							Tb/Ek	71237-3	$343
pIEx-2 Ek/LIC[3]		×		×	×		×			×							Tb/Ek	71240-3	$343
pIEx-3 Ek/LIC[3]		×		×	×		×			×						×	Tb/Ek	71245-3	$343
pIEx-7 Ek/LIC[3]		×			×†						×						Ek	71339-3	$343
pTriEx™-4 Ek/LIC[4]	×	×	×		×		×		×	×							Tb/Ek	70905-3	$350

* Components include Linearized Ek/LIC Vector; Control Insert; T4 DNA Polymerase, LIC-qualified; 10X T4 DNA Polymerase Buffer; DTT, EDTA, and dATP solutions; Nuclease-free Water; competent cells; SOC Medium; Test Plasmid

† with minimal extra amino acids

[1] T7*lac*-driven, tightly controlled, high-level expression in *E. coli*

[2] *polh*-driven, high-level baculovirus-mediated expression in insect cells

[3] For direct expression in insect cells with no virus infection

[4] *E. coli*, baculovirus, and mammalian systems

Accessory Products

Product	Size	Cat. No.	Price
T4 DNA Polymerase, LIC-qualified	250 U	70099-3	$209
NovaBlue GigaSingles™ Competent Cells	11 rxn	71227-3	$105
	22 rxn	71227-4	$204
Recombinant Enterokinase	50 U	69066-3	$93
100 mM IPTG Solution	15 ml	70527-3	$59
Overnight Express™ Autoinduction System 1	1 kit*	71300-3	$55
	1 kit**	71300-4	$220
Overnight Express Autoinduction System 2	1 kit*	71366-3	$98
	1 kit**	71366-4	$392

* includes enough reagents to induce 1 liter

** includes enough reagents to induce 5 liters

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KOD Hot Start DNA Polymerase* is a premixed complex of KOD HiFi DNA Polymerase and two monoclonal antibodies that inhibit the DNA polymerase and 3′→5′ exonuclease activities at ambient temperatures. KOD Hot Start combines the high fidelity, fast extension speed and outstanding processivity of KOD HiFi with the high specificity of an antibody-mediated hot start. KOD Hot Start DNA Polymerase generates blunt-ended PCR products suitable for cloning with Novagen LIC Vector Kits.

Features

- Highest accuracy, yield, and processivity among commercially available proofreading DNA polymerases
- Amplifies genomic DNA templates up to 12 kbp and plasmid and lambda DNA templates up to 21 kbp
- Successfully amplifies GC-rich sequences
- Eliminates mispriming and primer-dimer formation
- Convenient room-temperature setup compatible with automation
- Optimal KOD Hot Start Buffer for PCR performance over a wide range of targets

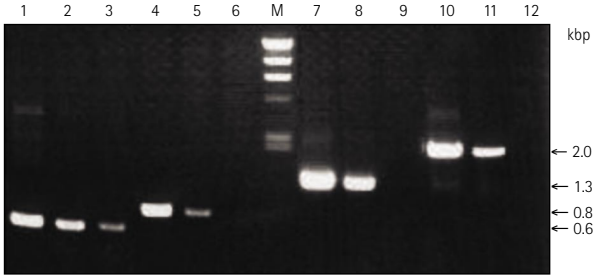
Product	Size	Cat. No.	Price
KOD Hot Start DNA Polymerase	200 U	71086-3	$173
	5 × 200 U	71086-4	$649

Components:

- 200 U or 5 × 200 U — KOD Hot Start DNA Polymerase (1.0 U/µl)
- 1.2 ml or 5 × 1.2 ml — 10X PCR Buffer for KOD Hot Start DNA Polymerase
- 1 ml or 5 × 1 ml — 25 mM $MgSO_4$
- 1 ml or 5 × 1 ml — dNTP mix (2 mM each)

FREE dNTP Mix included

Mizuguchi, H., Nakatsuji, M., Fujiwara, S., Takagi, M., and Imanaka, T. (1999) *J. Biochem. (Tokyo)* **126**, 762–768.



Lane(s)	Sample
M	λ/*Hin*d III DNA Markers
1, 4, 7, 10	Products of KOD Hot Start DNA Polymerase
2, 5, 8, 11	Products of *PfuTurbo* DNA Polymerase
3, 6, 9, 12	Products of *PfuUltra* high-fidelity DNA Polymerase

Performance of KOD Hot Start, *PfuTurbo*®, and *PfuUltra*™ high-fidelity DNA polymerases in amplification of human genomic DNA

The indicated DNA fragments were amplified using sets of specific primers and 100 ng human genomic DNA. PCR cycling parameters were as follows: initial denaturation at 95°C for 3 min; 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 2 min; and final extension at 72°C for 2 min. Reactions contained 0.3 µM each primer, the appropriate PCR buffer, 0.2 mM each dNTP, and 1 mM $MgSO_4$ (KOD Hot Start reactions) or approximately 2 mM $MgCl_2$ (*PfuTurbo* and *PfuUltra* reactions; included in 1X PCR buffer). Samples (equal volumes) were analyzed by agarose gel electrophoresis (1.2% TAE) and stained with ethidium bromide.

Note: 72°C is an optimal extension temperature for both Pfu enzymes but is suboptimal for KOD Hot Start genomic DNA amplification.

* Manufactured by TOYOBO and distributed by EMD Biosciences, Inc. Not available from EMD Biosciences, Inc. in Japan.

For more information or to order Novagen products, contact EMD Biosciences.

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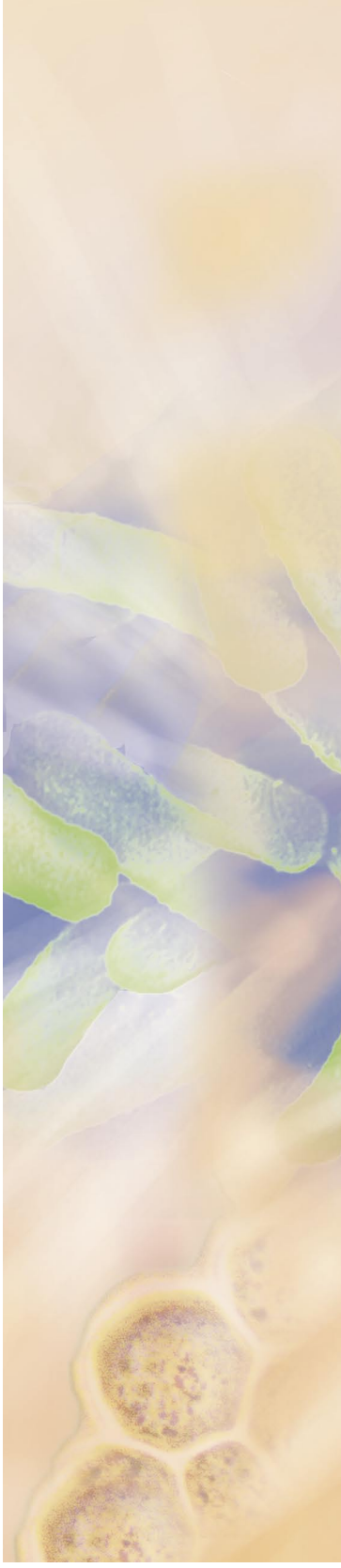

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