Duet Vectors

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About the Kits

pACYCDuet™-1 DNA	10 µg	71147-3
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pCOLADuet™-1 DNA	10 µg	71406-3

Description

Coexpression of multiple target genes in *E. coli* is advantageous for studying protein complexes. Coexpression often achieves optimal yield, solubility, and activity and may protect individual subunits from degradation (1–7). The Duet vectors are T7 promoter expression vectors, each designed to coexpress two target proteins in *E. coli*. The Duet vectors carry compatible replicons and antibiotic resistance markers and may be used together in appropriate host strains to coexpress up to eight proteins. Certain combinations of Duet vectors and pET or pETcoco™ vectors are also compatible for coexpression. The capability of Duet vectors to be cotransformed, propagated, and induced for robust target protein coexpression makes them ideal for the analysis of protein complexes (8, 9).

The Duet vectors are designed with compatible replicons (8–11) and drug resistance genes for effective propagation and maintenance of four plasmids in a single cell. pETDuet-1 carries the ColE1 replicon and *bla* gene (ampicillin resistance), pACYCDuet-1 carries the P15A replicon and *cat* gene (chloramphenicol resistance), and pCDFDuet-1 carries the CloDF13 replicon (12) and *aadA* gene (streptomycin/spectinomycin resistance). Two kanamycin-resistant Duet vectors are available; pRSFDuet-1 carries the RSF1030 replicon (13, 14) and pCOLADuet-1 carries the ColA replicon (15). Each vector carries two expression units each controlled by a T7*lac* promoter for high-level protein expression. Each promoter is followed by a ribosome binding site and multiple cloning site (MCS) region. A T7 terminator follows the second MCS. The multiple cloning regions have restriction sites that facilitate the cloning of two genes and the transfer from other Novagen pET constructs. The Duet vectors provide the option of producing native unfused proteins, or fusions to His•Tag® and S•Tag™ sequences for detection and purification of protein complexes.

Storage

Store DNA at –20°C or –70°C.

Multiple Cloning Sites and Fusion Tags

The Duet Vectors have two T7*lac* promoters, two MCS regions, and a single T7 terminator for the cloning and expression of two target open reading frames (ORFs). The plasmids also carry the *lacI* gene to ensure the expression of sufficient *lac* repressor to control basal expression. In all vectors, MCS1 has an *Nco* I restriction site at the ATG (Met) translation initiation site which can be used to produce unfused protein and has several restriction sites common to most pET vectors (*Bam*H I, *Eco*R I, *Sac* I, *Sal* I, *Hin*d III, and *Not* I) for easy transfer of clones. MCS1 also encodes an amino-terminal 6-amino acid (aa) His•Tag® sequence for detection and purification. In all vectors, MCS2 has an *Nde* I restriction site at the ATG (Met) translation initiation site, which can be used to produce unfused protein and several restriction sites (*Bgl* II, *Mun* I and *Xho* I) that generate overhangs compatible with *Bam*H I, *Eco*R I, and *Sal* I, respectively, for easy transfer of inserts in pET vectors. MCS2 also encodes an optional carboxy-terminal 15-aa S•Tag™ sequence for detection, purification, and quantification. The design of MCS regions facilitates the generation of two unfused proteins or one fusion protein with an N-terminal His•Tag, and/or one fusion protein with a C-terminal S•Tag, as desired for detection, purification, or quantification of protein complexes. Both MCS regions include 8-base pair (bp) rare cutting restriction enzymes, *Sse* 8387I and *Not* I in MCS1 and *Fse* I and *Sgf* I in MCS2, to facilitate the insertion of two ORFs into each vector.

Vector and Host Strain Compatibility

Vector compatibility

The vectors differ in their antibiotic resistance markers, replicons, and copy numbers.
Table 1 summarizes the antibiotic-resistant markers of the Duet Vectors.

Table 1 Duet Vector antibiotic resistance markers

Plasmid	Antibiotic resistance	Marker	Concentration*	Antibiotic Cat. No.
pETDuet™-1	ampicillin or carbenicillin	*bla*	50 µg/ml	Ampicillin; 171254 Carbenicillin; 69101-3
pACYCDuet™-1	chloramphenicol	*cat*	34 µg/ml	220551
pCDFDuet™-1	streptomycin or spectinomycin	*aadA*	50 µg/ml	Streptomycin; 5711 Spectinomycin; 567570
pRSFDuet™-1 pCOLADuet™-1	kanamycin		30 µg/ml	420311

*When cotransforming four Duet plasmids, the antibiotic concentrations should be reduced by half.

The various replicons carried by the Duet vectors, P15A (pACYCDuet-1), ColE1 (pETDuet-1), CloDF13 (pCDFDuet-1) are compatible with each other (9, 10)and with the replicons carried by the two kanamycin-resistant Duets, COLA (pCOLADuet-1), and RSF1030 (pRSFDuet-1). Duet vectors with different drug resistance markers can be used in combination for coexpression in the appropriate host strains. Duet vectors can also be used with the pET vectors and other constructs that have compatible replicons. Table 2 (page 4) summarizes the replicons and compatible replicons used in Novagen *E. coli* expression vectors and strains. The difference in target gene dosage attributed to plasmid copy number between any of the plasmids could be used to influence relative target protein expression levels.

Note:The combination of a "plain" T7 promoter pET plasmid [i.e., pET-3a-d, pET-20b(+), etc.] with a T7lac promoter plasmid is not recommended.

Determining the optimal combination of Duet vectors for the coexpression of any given set of ORFs is typically an empirical process, however, previous expression results may be useful when choosing expression constructs (9, 11). In general, the target ORFs expressed from pETDuet™-1 (ColE1 replicon), pACYCDuet™-1 (P15A replicon), and pCDFDuet™-1 (CloDF13 replicon) vectors were expressed at higher levels, when used in combination with pCOLADuet™-1 (COLA replicon) vector, than when used in combination with pRSFDuet™-1 (RSF1030 replicon) vector (11). In one experiment, it was found that in all 2-Duet vector combinations, and in three out of four 3-Duet vector combinations, the ORFs cloned on the higher copy pRSFDuet™-1 vector were observed to have the highest expression levels (9). Note, however, that expression of ORFs cloned into pRSFDuet-1 were substantially reduced in coexpression

experiments with pETDuet-1 and pACYCDuet-1 in the same cell. A significantly different pattern of expression was obtained when the lower copy number pCOLADuet™-1 was substituted for pRSFDuet-1 in an otherwise identical set of experiments (11). In this experiment, the expression of the ORFs encoded by pCOLADuet-1 were not substantially reduced when used in combination with both pACYCDuet-1 and pETDuet-1.

Table 2 Plasmid replicons and compatibility

Plasmid(s)	Replicon (source)	Copy number*	Compatible Replicons
pET (all), pETDuet-1	ColE1 (pBR322)	~40	P15A, Mini-F/RK2, CloDF13, RSF1030, ColA
pACYCDuet-1, pLysS, pLysE, pLacI, pRARE, pRARE-2	P15A (pACYC184)	10–12	ColE1, Mini-F/RK2, CloDF13, RSF1030, ColA
pCDFDuet™-1, pCDF	CloDF13	20–40	ColE1, P15A, RSF1030, ColA
pRSFDuet-1, pRSF	RSF1030	> 100	ColE1, P15A, CloDF13
pCOLADuet™-1	COLA (ColA)	20–40	ColE1, P15A, CloDF13, Mini-F/RK2
pETcoco™ (all)	Mini-F/RK2 (pBeloBAC11, RK2)	amplifiable to ~40	ColE1, P15A , ColA

* Copy number was estimated based on gel analysis (9, 16)

Host strain compatibility

For protein production, the Duet recombinants are transferred to an *E. coli* expression host (DE3) containing a chromosomal copy of the gene for T7 RNA polymerase. The choice of expression host strain is based on strain characteristics and expression vector compatibility. Review the Competent Cells Protocol (User Protocol TB009) for complete descriptions of the host strain characteristics. Use the following tables to determine compatibility. Compatible vectors and host strains are listed in Table 3 below. For expression host strain group, see Table 4 (page 6). For compatible combinations with pETcoco™ plasmid, please consult User Protocol TB333.

Note: The pETcoco vectors are not compatible with pCDFDuet-1 or pRSFDuet-1.

Table 3 Vector and host strain compatibility

Compatible Vector Combinations				Number of coexpressed target proteins	Compatible expression host strains
Vector 1	**Vector 2**	**Vector 3**	**Vector 4***		
pETDuet™-1 (Amp^R)	pACYCDuet™-1 (Cam^R)	pRSFDuet™-1 or pCOLADuet™-1 (Kan^R)	pCDFDuet™-1 (Sm^R)	8	Group A
pETDuet-1 (Amp^R)	pRSFDuet-1or pCOLADuet-1 (Kan^R)	pCDFDuet-1 (Sm^R)		6	Group C
pETDuet-1 (Amp^R)	pACYCDuet-1 (Cam^R)	pRSFDuet-1 or pCOLADuet-1 (Kan^R)		6	Group A
pETDuet-1 (Amp^R)	pACYCDuet-1 (Cam^R)	pCDFDuet-1 (Sm^R)		6	Group B
pRSFDuet-1 or pCOLADuet-1 (Kan^R)	pCDFDuet-1 (Sm^R)	pACYCDuet-1 (Cam^R)		6	Group A
pETDuet-1 (Amp^R)	pRSFDuet-1 or pCOLADuet-1 (Kan^R)			4	Group C
pETDuet-1 (Amp^R)	pCDFDuet-1 (Sm^R)			4	Group D
pETDuet-1 (Amp^R)	pACYCDuet-1 (Cam^R)			4	Group B
pRSFDuet™-1 or pCOLADuet-1 (Kan^R)	pCDFDuet-1 (Sm^R)			4	Group C
pACYCDuet-1 (Cam^R)	pRSFDuet™-1 or pCOLADuet-1 (Kan^R)			4	Group A
pACYCDuet-1 (Cam^R)	pCDFDuet-1 (Sm^R)			4	Group B

Amp; ampicillin/carbenicillin, 50 µg/ml: Kan; kanamycin, 30 µg/ml: Cam; chloramphenicol, 34 µg/ml:
Sm; streptomycin/spectinomycin, 50 µg/ml
*When cotransforming four Duet plasmids, the antibiotic concentrations should be reduced by half.

Table 4 Vector and Host Strain Compatibility

Vector	Compatible expression host strains
pETDuet-1	Group D
pACYCDuet-1	Group B
pCDFDuet-1	Group D
pRSFDuet-1 or pCOLADuet-1	Group C

Table 4 Strain groups

Group A	Group B	Group C	Group D
B834(DE3)	B834(DE3)	B834(DE3)	B834(DE3)
BL21(DE3)	BL21(DE3)	B834(DE3)pLysS[2]	B834(DE3)pLysS
BLR(DE3)	BLR(DE3)	BL21(DE3)	BL21(DE3)
HMS174(DE3)	HMS174(DE3)	BL21(DE3)pLysS[2]	BL21(DE3)pLysS
NovaBlue(DE3)	NovaBlue(DE3)	BLR(DE3)	BLR(DE3)
Origami™ 2(DE3)[1]	Origami(DE3)[1]	BLR(DE3)pLysS[2]	BLR(DE3)pLysS
Tuner™(DE3)	Origami 2(DE3)[1]	HMS174(DE3)	HMS174(DE3)
	Origami B(DE3)	HMS174(DE3)pLysS[2]	HMS174(DE3)pLysS
	Tuner(DE3)	NovaBlue(DE3)	NovaBlue(DE3)
		Origami 2(DE3)[1]	Origami(DE3)[1]
		Origami 2(DE3)pLysS[1, 2]	Origami(DE3)pLysS[1]
		Rosetta™(DE3)[2]	Origami 2(DE3)[1]
		Rosetta(DE3)pLysS[2]	Origami 2(DE3)pLysS[1]
		Rosetta 2(DE3)[2]	Origami B(DE3)
		Rosetta 2(DE3)pLysS[2]	Origami B(DE3)pLysS
		RosettaBlue™(DE3)[2]	Rosetta(DE3)
		RosettaBlue(DE3)pLysS[2]	Rosetta(DE3)pLysS
		Rosetta-gami™ 2(DE3)[1, 2]	Rosetta 2(DE3)
		Rosetta-gami 2(DE3)pLysS[1, 2]	Rosetta 2(DE3)pLysS
		Tuner(DE3)	RosettaBlue(DE3)
		Tuner(DE3)pLysS[2]	RosettaBlue(DE3)pLysS
			Rosetta-gami(DE3)[1]
			Rosetta-gami(DE3)pLysS[1]
			Rosetta-gami 2(DE3)[1]
			Rosetta-gami 2(DE3)pLysS[1]
			Rosetta-gami B(DE3)
			Rosetta-gami B(DE3)pLysS[1]
			Tuner(DE3)
			Tuner(DE3)pLysS

1.) These strains carry a mutation in ribosomal protein (*rpsL*) conferring resistance to streptomycin; therefore streptomycin is not necessary to maintain strain genotype. If using pCDF vectors, spectinomycin must be used for antibiotic selection because *rpsL* mutation confers streptomycin resistance.

2.) These strains carry a plasmid with a P15A replicon; when used with pETDuet™-1 and pRSFDuet™-1 for coexpression substantially reduced expression levels of ORFs cloned into pRSFDuet-1 may result. This reduction in coexpression has not been observed when pCOLADuet™-1 is substituted for pRSFDuet-1. See page 4 for more information.

Procedures

Cloning

Cloning two ORFs into the same plasmid requires some extra planning. When creating two-ORF constructs, the first insert should lack restriction sites that will be used to insert the second ORF. Typically, the first insert is cloned and an intermediate plasmid is isolated and verified. Then the second ORF is inserted into the remaining MCS to generate the final construct that also requires verification. Unique restriction sites can be added to the second ORF by PCR amplification with primers that contain the desired restriction sites (17, 18)We recommend the use of the robust, high-fidelity KOD HiFi, Hot Start, or XL DNA polymerases, which greatly reduce the chance of generating PCR-based mutations. Standard cloning procedures, including vector and insert preparation and ligation reactions, can be found in the pET System Manual (User Protocol TB055). A high efficiency *recA*$^{-}$, *endA*$^{-}$ host strain such as NovaBlue (Cat. No. 70181) should be used for cloning.

Note: For greatest specificity use KOD Hot Start DNA Polymerase (Cat. No. 71086-3). For greatest yield of long complex targets, use KOD XL DNA Polymerase(Cat. No. 71087-3). (See User Protocol TB341 and TB342, respectively).

Analysis of Duet recombinants

Plasmid DNA from candidate recombinants should be verified for the presence of the correct insert and reading frame. Verification should occur prior to cotransformation to isolate and analyze a single plasmid clone. Several methods available for analysis of transformants include colony PCR, plasmid preparation, restriction analysis, sequencing, and *in vitro* transcription and translation. These methods are described in the pET System Manual (User Protocol TB055).

Duet plasmid DNA can be isolated for transformation into expression hosts, restriction mapping, *in vitro* transcription/translation, and sequence analysis. Isolate pETDuet™-1, pACYCDuet™-1, pCOLADuet™-1, and pCDFDuet™-1 plasmids using low-copy number DNA purification methods, as found in scientific literature. For pRSFDuet™-1 plasmids, use the high-copy number protocol provided. Purified DNA must be RNase-free. Plasmid DNA isolated using SpinPrep™ Plasmid Kits, or protocols from scientific literature may require an additional phenol:CIAA extraction (1:1; CIAA is 24 parts chloroform, 1 part isoamyl alcohol) to eliminate RNases (described in the pET System Manual). Use the table below to determine an appropriate plasmid preparation kit.

Sequencing primers

The following table lists appropriate primers to use for PCR and sequence analysis. Note that because the Duet vectors have two T7*lac* promoters each, the T7 Promoter Primer is not appropriate for PCR or sequence analysis.

MCS	Primer Type	pACYCDuet™-1, pCDFDuet™-1, pRSFDuet™-1, pCOLADuet™-1	pETDuet™-1
MCS1	Sense	pACYCDuetUP1 Primer Cat. No. 71178-3	pET Upstream Primer Cat. No. 69214-3
	Antisense	DuetDOWN-1 Primer Cat. No. 71179-3	DuetDOWN-1 Primer Cat. No. 71179-3
MCS2	Sense	DuetUP2 Primer Cat. No. 71180-3	DuetUP2 Primer Cat. No. 71180-3
	Antisense	T7 Terminator Primer Cat. No. 69337-3	T7 Terminator Primer Cat. No. 69337-3

Transformation into expression host strains

Follow the protocols provided in User Protocol TB009 (see User Protocol TB333 if cotransforming with pETcoco™ vectors) for the transformation of Duet vectors into competent cells. For transformations into expression strains using supercoiled plasmid, add 1 µl containing 10–40 ng of each plasmid into competent cells. Perform a 1 h outgrowth prior to plating. Plate 10–70 µl of the transformation mixture. When cotransforming four Duet plasmids, plate the entire transformation mixture, using several plates if necessary and reduce antibiotic concentration by half. Note that antibiotics appropriate for all vectors must be included in the plates and media when cotransforming multiple vectors. Use the tables on pages 5–6 to determine which expression hosts are appropriate for any combination of expression vectors.

Induction

After the plasmids are established in a λDE3 lysogen, expression of the target ORF can be induced by using medium prepared with Overnight Express™ Autoinduction System components (19), or by adding IPTG to a conventional medium. Medium produced with Overnight Express components directs high-density cell growth in the absence of induction followed by autoinduction during the overnight incubation (see User Protocol TB383 for more information). If using IPTG for induction, a final concentration of 1 mM IPTG should be added when the cells reach an OD_{600} of 0.6. Induce for 3 h. Follow the induction protocols in the pET System Manual (User Protocol TB055). See User Protocol TB333 if using a pETcoco™ recombinant with pETDuet or pACYCDuet vectors.

Induction analysis and protein detection, purification, and quantification

For recommendations and protocols regarding induction analysis and optimization, and sample preparation, purification, detection, and quantification, review the pET System Manual (User Protocol TB055) and the following Technical Bulletins, as appropriate.

Coexpression experiments may result in different expression levels of target proteins (9, 20) These differences may be due to unique translation rates or unequal copy numbers for the two expression plasmids (21). If dissimilar expression levels were caused by unequal plasmid copy number, cloning the target genes into the same plasmid may alleviate this problem.

Detection/Assay Tools for Fusion Tags

His•Tag® detection	**Cat. No.**	**Size**	**User Protocol No./Applications**
His•Tag Monoclonal Antibody	70796-4 70796-3	3 µg 100 µg	TB283 immunofluorescence, immunoprecipitation, Western blotting
His•Tag AP Western Reagents	70972-3	25 blots	TB283 colorimetric detection
His•Tag AP LumiBlot™ Reagents	70973-3	25 blots	TB283 chemiluminescent detection
His•Tag HRP LumiBlot Reagents	70974-3	25 blots	TB283 chemiluminescent detection
S•Tag™ detection	**Cat. No.**	**Size**	**User Protocol No./Applications**
S-protein AP Conjugate	69598-3	50 µl	TB097 Western blotting
S-protein HRP Conjugate	69047-3	50 µl	TB136 Western blotting
Biotinylated S-protein	69218-3	250 µl	Western blotting
S-protein FITC Conjugate	69060-3	200 µl	TB143 immunofluorescence
S•Tag AP Western Blot Kit	69213-3	25 blots	TB082 colorimetric detection
S•Tag AP LumiBlot Kit	69099-3	25 blots	TB164 chemiluminescent detection
S•Tag HRP LumiBlot Kit	69058-3	25 blots	TB145 chemiluminescent detection
Quantitative assay	**Cat. No.**	**Size**	**User Protocol No./Sensitivity**
FRETWorks™ S•Tag Assay Kit	70724-3 70724-4	100 assays 1000 assays	TB251 fluorescent assay, Limit < 1 fmol
S•Tag Rapid Assay Kit	69212-3	100 assays	TB082 Limit 20 fmol
Western blot protein markers	**Cat. No**	**Size**	**User Protocol No./Size standards**
Perfect Protein™ Western Markers	69959-3	25 lanes	TB102; 15, 25, 35, 50, 75, 100 and 150 kDa
Trail Mix™ Western Markers	70982-3	25 lanes	TB310; 15, 25, 35, 50, 75, 100 and 150 kDa, and 15, 16, 100 kDa prestained markers
Extraction reagents	**Cat. No.**	**Size**	**User Protocol No./Capacity and features**
BugBuster® Protein Extraction Reagent	70584-3 70584-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. Tris-buffered
BugBuster HT Protein Extraction Reagent	70922-3 70922-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. Tris-buffered and pre-mixed with Benzonase® Nuclease
BugBuster Master Mix	71456-3 71456-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. Tris-buffered and pre-mixed with Benzonase and rLysozyme™ Solution
BugBuster 10X Protein Extraction Reagent	70921-3 70921-4 70921-5	10 ml 50 ml 100 ml	TB245 Dilute to 1X with choice of buffer and use 5 ml/g wet cell paste
BugBuster (primary amine-free) Extraction Reagent	70923-3 70923-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. PIPPS-buffered
PopCulture® Reagent	71092-3 71092-4 71092-5	15 ml 75 ml 250 ml	TB323 Use 0.1 volume per ml of culture
rLysozyme Solution	71110-3 71110-4 71110-5	300 KU 1200 KU 6000 KU	TB334 and TB323 Use 40 U per ml of culture volume with PopCulture Reagent and 1 KU per ml of BugBuster Reagent

Extraction Reagents	Cat. No.	Size	User Protocol No./Capacity and Features
Benzonase® Nuclease, Purity > 90%	70746-3 70746-4	10,000 U 2,500 U	TB245, 323, 261; Use 25 U per ml original culture volume with PopCulture® and BugBuster® Reagent
Lysonase™ Bioprocessing Reagent	71230-3 71230-4 71230-5	0.2 ml 1 ml 5 ×1 ml	TB361 Optimized blend of rLysozyme™ Solution and Benzonase Nuclease. Use 3 µl per ml lysis buffer
His•Tag® purification	**Cat. No.**	**Size**	**User Protocol No./Capacity and Features**
Ni-NTA His•Bind® Resin	70666-3 70666-4 70666-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin
Ni-NTA Superflow	70691-3 70691-4 70691-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin; high flow rates and pressures
Ni-NTA Buffer Kit	70899-3		TB273 All buffers for native purification using Ni-NTA His•Bind and Ni-NTA Superflow resins
His•Bind Resin	69670-3 69670-4 69670-5	10ml 50 ml 100ml	TB054 Capacity is 8 mg/ml settled resin
His•Bind Buffer Kit	69755-3		TB054 All buffers for native purification using His•Bind Resin
His•Bind Columns	70971-3 70971-4	pkg/5 pkg/25	TB054 pre-packed, pre-charged; Capacity is 10 mg per column
His•Mag™ Agarose Beads	71002-3 71002-4	2 ml 10 ml	TB054 magnetic agarose beads, pre-charged; Capacity is 5 mg per ml settled beads
His•Bind Purification Kit	70239-3		TB054 10 ml His•Bind Resin, Buffers and Chromatography Columns
BugBuster Ni-NTA His•Bind Purification Kit	70751-3		TB273 10 ml Ni-NTA His•Bind Resin, BugBuster, Benzonase, and Chromatography Columns
BugBuster His•Bind Purification Kit	70793-3		TB054 10 ml His•Bind Resin and Buffer, BugBuster, Benzonase, and Chromatography Columns
PopCulture His•Mag Purification Kit	71114-3		TB054 Process 40 × 3 ml cultures purifying up to 375 µg per 3 ml culture
RoboPop™ His•Mag Purification Kit	71103-3		TB327 Purify up to 12 mg per 96 wells
RoboPop Ni-NTA His•Bind Kit	71188-3		TB346 Purify up to 96 mg per 96 wells
S•Tag™ purification	**Cat. No.**	**Size**	**User Protocol No./Capacity and Features**
S-protein Agarose	69704-3 69704-4	2 ml 5 × 2 ml	TB087, TB160; Purify up to 1 mg per 2 ml settled resin
S•Tag Thrombin Purification Kit	69232-3		TB087 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)
S•Tag rEK Purification Kit	69065-3		TB160 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)

References

1. Sanchez, A., Trappier, S.G., Stroher, U., Nichol, S.T., Bowen, M.D., and Feldmann, H. (1998) *Virology* **240**, 138.
2. Stebbins, C.E., Kaelin, W.G., Jr., and Pavletich, N.P. (1999) *Science* **284**, 455–461.
3. Shuman, S. (1990) *J. Biol. Chem.* **265**, 11960–11966.
4. Li, C., Schwabe, J.W., Banayo, E., and Evans, R.M. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 2278–2283.
5. Ishiai, M., Sanchez, J.P., Amin, A.A., Murakami, Y., and Hurwitz, J. (1996) *J. Biol. Chem.* **271**, 20868–20878.
6. Henricksen, L.A., Umbricht, C.B., and Wold, M.S. (1994) *J. Biol. Chem.* **269**, 11121–11132.
7. Tan, S. (2001) *Protein Expr. Purif.* **21**, 224–234.
8. Novy, R., Yaeger, K., Held, D., and Mierendorf, R. (2002) *in**Nova**tions* **15**, 2–6.
9. Held, D., Yaeger, K., and Novy, R. (2003) *in**Nova**tions* **18**, 4–6.
10. Selzer, G., Som, T., Itoh, T., and Tomizawa, J. (1983) *Cell* **32**, 119–129.
11. Held, D., Yaeger, K., and Novy, R. (2004) *in**Nova**tions* **19**, 17–19.
12. Nijkamp, H.J., de Lang, R., Stuitje, A.R., van den Elzen, P.J., Veltkamp, E., and van Putten, A.J. (1986) *Plasmid* **16**, 135–160.
13. Som, T. and Tomizawa, J. (1982) *Mol. Gen. Genet.* **187**, 375–383.
14. Cannon, P.M. and Strike, P. (1992) *Plasmid* **27**, 220–230.
15. Zverev, V.V. and Khmel, I.A. (1985) *Plasmid* **14**, 192–199.
16. Sektas, M. and Szybalski, W. (2002) *in**Nova**tions* **14**, 6–8.
17. Novy, R. (1999) *in**Nova**tions* **10**, 13.
18. Novy, R., Kolb, K., and Mierendorf, R. (1999) *in**Nova**tions* **9**, 6–8.
19. Rucker, P., Torti, F.M., and Torti, S.V. (1997) *Protein Eng.* **10**, 967–973.
20. Grabski, A., Drott, D., and Mehler, M. (2003) *in**Nova**tions* **16**, 11–13.
21. Tsao, K.L. and Waugh, D.S. (1997) *Protein Expr. Purif.* **11**, 233–240.

Bacterial Strain Non-distribution Agreement

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2. Gene clones and libraries in the Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B host strains may be distributed for research purposes only, provided that the recipient acknowledge the foregoing condition.
3. Commercial customers must obtain a research license agreement from Brookhaven Science Associates before purchasing and using DE3 lysogens of host strains Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B.

The initial purchaser may refuse to accept the above conditions by returning the kit unopened and the enclosed materials unused. By accepting or using the kit or the enclosed materials, you agree to be bound by the foregoing conditions.