Duet Vectors

Table of Contents

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About the Kits

pACYCDuet™-1 DNA	10 µg	71147-3
pETDuet™-1 DNA	10 µg	71146-3
pCDFDuet™-1 DNA	10 µg	71340-3
pRSFDuet™-1 DNA	10 µg	71341-3
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.

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 T7*lac *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, pACYCDuet™-1, and pCDFDuet™-1 vectors were expressed at higher levels, when used in combination with pCOLADuet™-1 vector, than when used in combination with pRSFDuet™-1 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	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
pET Duet	Group D
pACYC Duet	Group B
pCDF Duet	Group D
pRSF Duet or pCOLA Duet	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	B834(DE3)pLysS
BLR(DE3)	BLR(DE3)	BL21(DE3)	BL21(DE3)
HMS174(DE3)	HMS174(DE3)	BL21(DE3)pLysS	BL21(DE3)pLysS
NovaBlue(DE3)	NovaBlue(DE3)	BLR(DE3)	BLR(DE3)
Origami™ 2(DE3)*	Origami(DE3)*	BLR(DE3)pLysS	BLR(DE3)pLysS
Tuner™(DE3)	Origami 2(DE3)*	HMS174(DE3)	HMS174(DE3)
	Origami B(DE3)	HMS174(DE3)pLysS	HMS174(DE3)pLysS
	Tuner(DE3)	NovaBlue(DE3)	NovaBlue(DE3)
		Origami 2(DE3)*	Origami(DE3)*
		Origami 2(DE3)pLysS*	Origami(DE3)pLysS*
		Rosetta™(DE3)	Origami 2(DE3)*
		Rosetta(DE3)pLysS	Origami 2(DE3)pLysS*
		Rosetta 2(DE3)	Origami B(DE3)
		Rosetta 2(DE3)pLysS	Origami B(DE3)pLysS
		RosettaBlue™(DE3)	Rosetta(DE3)
		RosettaBlue(DE3)pLysS	Rosetta(DE3)pLysS
		Rosetta-gami™ 2(DE3)*	Rosetta 2(DE3)
		Rosetta-gami 2(DE3)pLysS*	Rosetta 2(DE3)pLysS
		Tuner(DE3)	RosettaBlue(DE3)
		Tuner(DE3)pLysS	RosettaBlue(DE3)pLysS
			Rosetta-gami(DE3)*
			Rosetta-gami(DE3)pLysS*
			Rosetta-gami 2(DE3)*
			Rosetta-gami 2(DE3)pLysS*
			Rosetta-gami B(DE3)
			Rosetta-gami B(DE3)pLysS
			Tuner(DE3)
			Tuner(DE3)pLysS

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

Procedures

Cloning

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

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.

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. When isolating pETDuet™-1, pACYCDuet™-1, pCOLADuet™-1, and pCDFDuet™-1 plasmids with Mobius™ or UltraMobius™ kits, use the low-copy number protocol provided in the Mobius User Protocols. For pRSFDuet™-1 plasmids, use the high-copy number protocol provided. Plasmid DNA isolated with Mobius or UltraMobius kits is essentially RNase-free. However, plasmid DNA isolated with SpinPrep™ Plasmid Kits or kits from other manufacturers 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.

Plasmid Preparation Kit	Culture size	DNA Yield	Cat. No.	Size
Mobius 1000 Plasmid Kit	100 ml (high-copy) 250 ml–1.5 L (low-copy)	> 1 mg (high-copy) 200 µg–1 mg (low-copy)	70854-3 70853-3 70853-4	2 rxn* 10 rxn* 25 rxn*
UltraMobius 1000 Plasmid Kit	100 ml (high-copy) 250 ml–1.5 L (low-copy)	> 1 mg (high-copy) 200 µg–1 mg (low-copy)	70907-3 70906-3 70906-4	2 rxn* 10 rxn* 25 rxn*
Mobius 500 pET Plasmid Kit	500 ml culture	500 µg (low-copy)	70969-3	10 rxn
Mobius 200 Plasmid Kit	35 ml culture (high-copy or low-copy)	> 200 µg (high-copy) > 30 µg (low-copy)	70970-3	25 rxn
UltraMobius 200 Plasmid Kit	35 ml culture (high-copy or low-copy)	> 200 µg (high-copy) > 30 µg (low-copy)	71090-3	25 rxn
SpinPrep Plasmid Kit	1–3 ml culture	5–10 µg (high-copy) 0.25–1 µg (low-copy)	70957-3 70851-3	20 rxn 100 rxn

*The kit sizes described are for the 100-ml (high-copy) or 250-ml (low-copy) preparations. Additional buffers are required for > 250-ml (low-copy) scale (User Protocol TB279).

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. 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 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•Bind Quick Columns	70159-3 70159-4	pkg/12 pkg/60	TB054 pre-packed, pre-charged; requires vacuum, Capacity is 5 mg per column
His•Bind Quick 300 Cartridges	70155-3 70155-4	pkg/10 pkg/50	TB054 pre-packed, pre-charged; Capacity is 0.5 mg per cartridge
His•Bind Quick 900 Cartridges	70153-3 70153-4	pkg/10 pkg/50	TB054 pre-packed, pre-charged; Capacity is 2 mg per cartridge
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 Quick Buffer Kit	70665-3		TB054 all buffers for native purification using His•Bind Columns, Quick Columns, Cartridges and His•Mag Agarose Beads; No charge buffer included
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)

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The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates (BSA). This technology, including bacteria, phages, and plasmids that carry the gene for T7 RNA polymerase, is made available on the following conditions:

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2. No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this assurance letter and agrees to be bound by its terms. This limitation applies to any of the following materials that are included in this kit and to any derivatives you may make of them:

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E. coli BL21(DE3)pLysS	*E. coli* Origami B(DE3)	*E. coli* Rosetta-gami 2(DE3)
E. coli BL21(DE3)pLysE	*E. coli* Origami B(DE3)pLysS	*E. coli* Rosetta-gami 2(DE3)pLysS
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E. coli Origami(DE3)pLysS	*E. coli* RosettaBlue(DE3)pLysS	Bacteriophage λDE3
E. coli Origami(DE3)pLacI	*E. coli* RosettaBlue(DE3)pLacI	

3. 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.

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