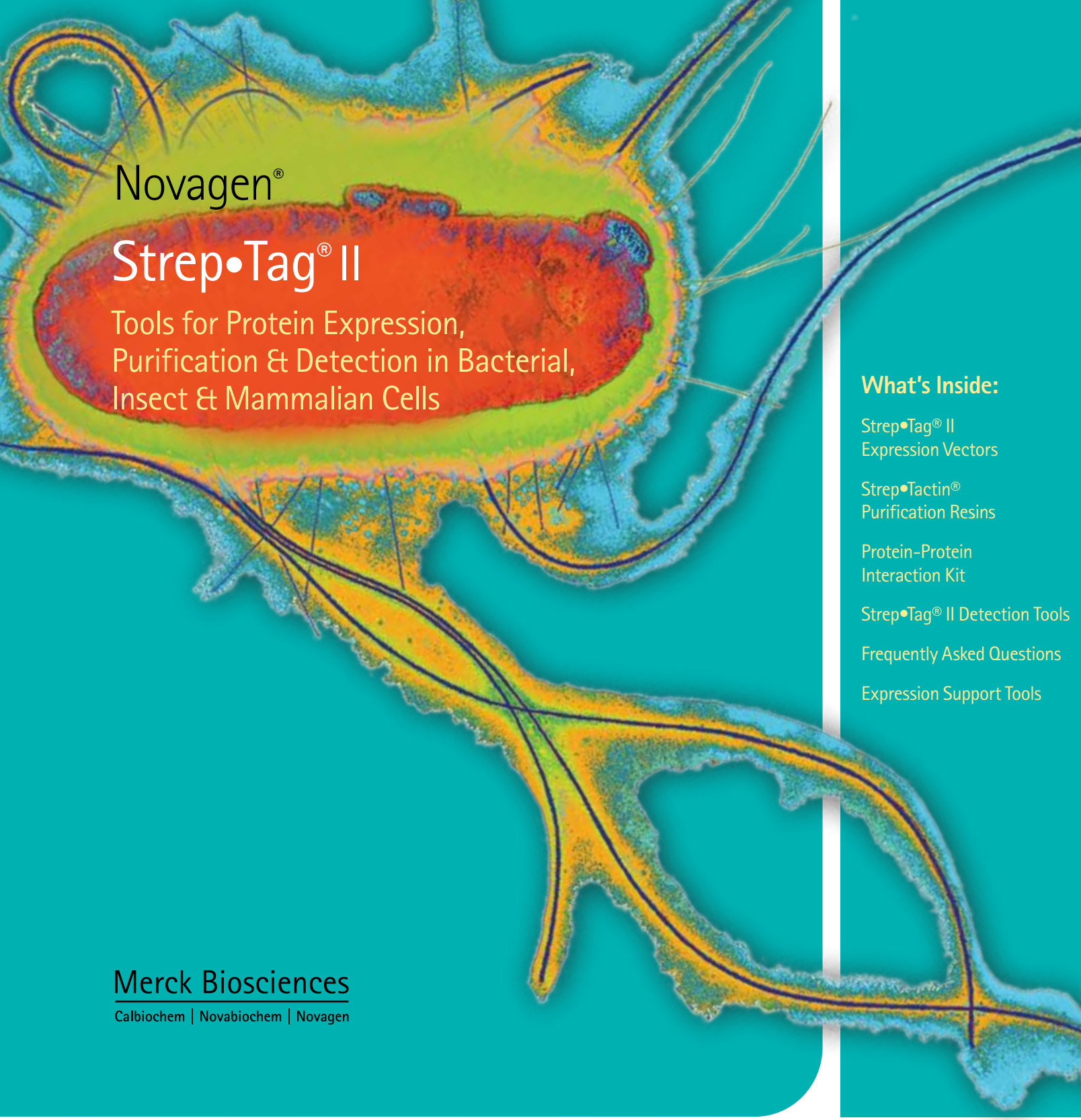

Novagen®

Strep•Tag® II

Tools for Protein Expression, Purification & Detection in Bacterial, Insect & Mammalian Cells

What's Inside:

Merck Biosciences

Calbiochem | Novabiochem | Novagen

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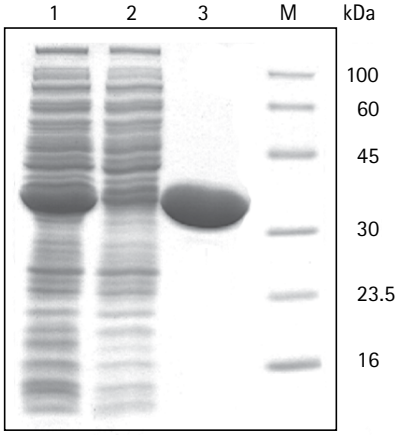
Introduction

To facilitate simple purification and easy detection of recombinant proteins, open reading frames are commonly cloned into expression vectors engineered to generate affinity-tagged fusion proteins. Although these purification methods are fast and convenient, many do not result in protein purity levels necessary for downstream applications and making a secondary purification step necessary. Strep•Tag® technology, using the Strep•Tag II fusion tag, is based on the reliable biotin/streptavidin binding specificity. The small Strep•Tag II sequence is an 8-amino acid fusion tag with binding specificity comparable to biotin. The small size of the tag reduces potential interference with target protein structure or function. The tag binds to Strep•Tactin® protein, which is an engineered streptavidin with an optimized Strep•Tag II binding site. Strep•Tag II binds to Strep•Tactin protein nearly 100 times tighter than it binds to streptavidin, but elutes under gentle, physiological conditions and the resin can be regenerated for reuse. Rapid, one-step affinity purification can result in active fusion proteins of greater than 95% purity. The Strep•Tag system includes a selection of Strep•Tag II vectors for expression in bacterial, insect, or mammalian cells; a wide range of Strep•Tactin affinity purification resins and buffers; and Strep•Tag II detection reagents. Further, the system allows protein:protein complexes to be isolated in a single step.

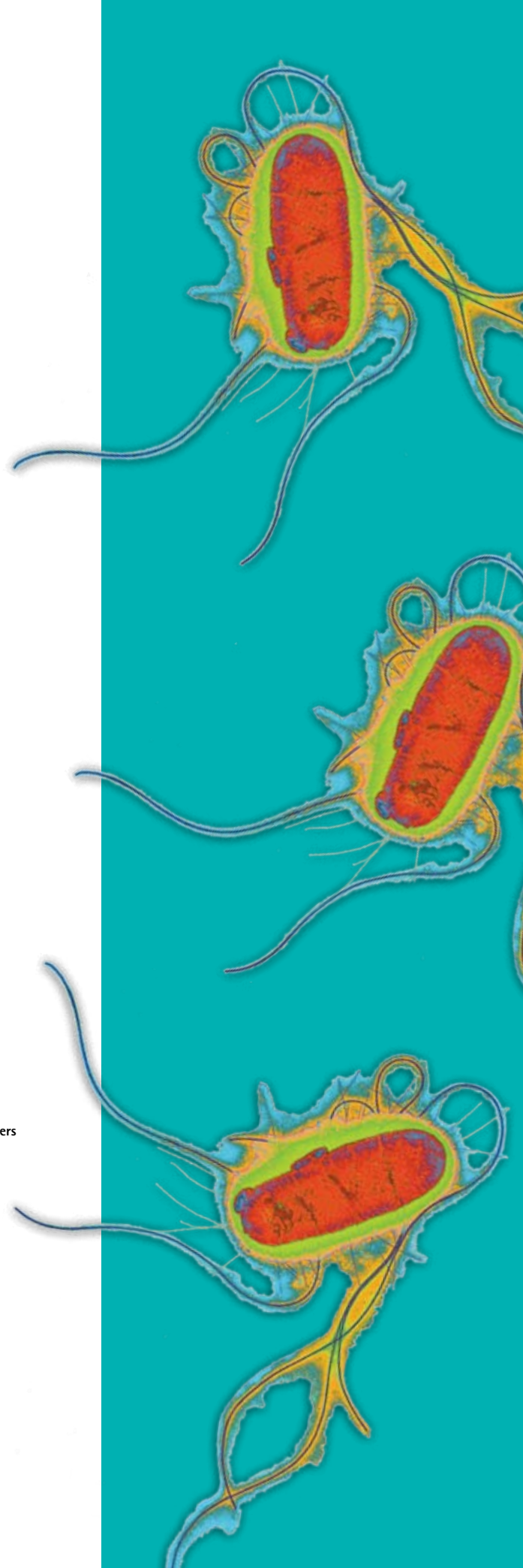
Features:

- Express target proteins in pET, InsectDirect™, and pTriEx™ Systems
- Achieve >95% purity in one purification step
- Elute bioactive proteins under physiological conditions
- Purify intact protein complexes



Lane	Sample
1	Total cell protein
2	Flow-through
3	Purified Strep•Tag II fusion protein
M	Strep•Tag II Perfect Protein™ Markers

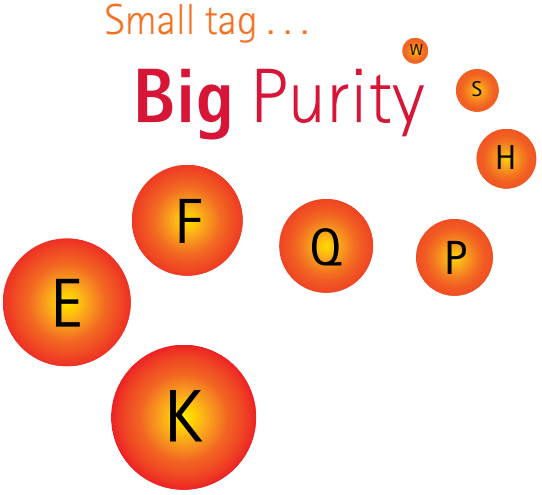
Purification of a 36-kDa enzyme expressed as a Strep•Tag II fusion in *E. coli*
The cells were harvested and extracts prepared. Samples were taken to assess total cell protein. The remaining extract was loaded onto a Strep•Tactin column and the Strep•Tag II fusion protein was purified by gravity flow using physiological buffer conditions (100 mM Tris-HCl, pH 8.0) according to the standard protocol. Samples of total cell protein, flow-through, and purified fractions were analyzed by SDS-PAGE.



 For more information or to place an order, contact your local office (see back cover).

Strep•Tag® II Fusion Tag

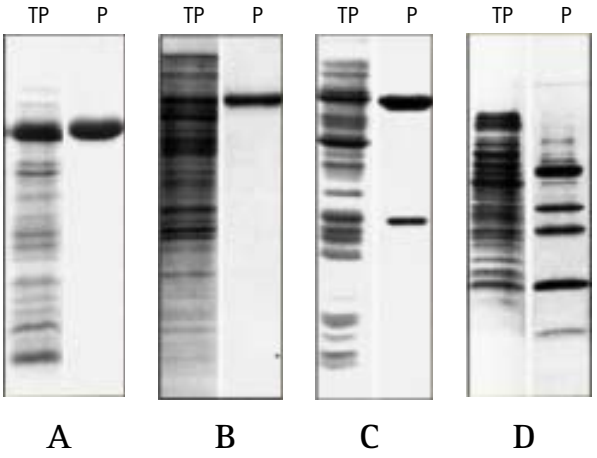
The small Strep•Tag® II peptide consists of only eight amino acids (WSHPQFEK), yet its binding specificity for Strep•Tactin® protein is comparable to that of biotin, although with reduced affinity enabling gentle elution. Due to the size and composition of the peptide, the Strep•Tag II peptide has negligible effect on most recombinant proteins. Typically, it does not interfere with the folding or bioactivity of the recombinant protein, making it an ideal fusion tag for affinity purification and detection. In many cases, there is no need to remove the tag after purification.



Strep•Tactin® Protein

Strep•Tactin® protein, a derivative of streptavidin, has an optimized binding pocket that is specific for the Strep•Tag II peptide. It is a stable protein that has chemical properties similar to those of streptavidin, making it compatible with a broad range of detergents, chelators, salt, and redox conditions. Strep•Tactin resins can be reused 3-5 times without any loss in performance.

Examples of active proteins purified in one step



TP: Total cell protein
P: Purified protein

A: **Metalloenzyme:** *E. coli* alkaline phosphatase.

B: **Large protein:** Oat phytochrome A, 120 kDa.

C: **A heterodimeric protein:** *Helicobacter pylori* **urease;** copurification of the heavy chain with the light chain fused to the Strep•Tag II peptide.

D: **Multimeric membrane protein:** An *E. coli* periplasmic fraction containing a Strep•Tag II Fv fragment with an affinity for cytochrome c oxidase was combined with a crude *Paracoccus denitrificans* membrane preparation. Subsequently, four subunits of cytochrome c oxidase were copurified with the Strep•Tag II Fv fragment.

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Expression Vectors

Vector	Size	Cat. No.	Price
pET-51b(+) DNA	10 µg	71553-3	
pET-51 Ek/LIC Vector Kit	20 rxn	71570-3	
pET-52b(+) DNA	10 µg	71554-3	
pET-52 3C/LIC Vector Kit	20 rxn	71571-3	
pIEx™-8 DNA	20 µg	71555-3	
pIEx-8 Ek/LIC Vector Kit	20 rxn	71572-3	
pIEx-9 DNA	20 µg	71556-3	
pIEx-9 3C/LIC Vector Kit	20 rxn	71573-3	
pIEx-10 DNA	20 µg	71557-3	
pIEx-10 Ek/LIC Vector Kit	20 rxn	71574-3	
pTriEx™-5 DNA	20 µg	71558-3	
pTriEx-5 Ek/LIC Vector Kit	20 rxn	71575-3	
pTriEx-6 DNA	20 µg	71559-3	
pTriEx-6 3C/LIC Vector Kit	20 rxn	71576-3	
pTriEx-7 DNA	20 µg	71560-3	
pTriEx-7 Ek/LIC Vector Kit	20 rxn	71577-3	

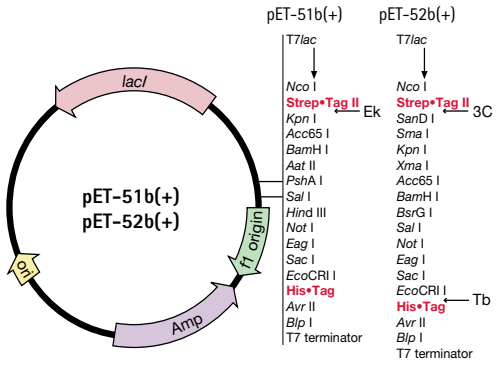
NEW Strep•Tag® II vectors

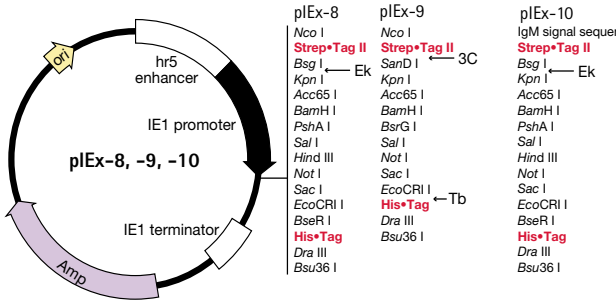
The latest pET (*E. coli*), pIEx™ (insect) and pTriEx™ (multi-system: *E. coli*, baculovirus, and mammalian) expression vectors incorporate the Strep•Tag® II fusion tag. The short 8-amino acid Strep•Tag II coding sequence is added as an N-terminal fusion tag, just upstream of an enterokinase (Ek) or HRV 3C (3C) cleavage site. These expression vectors share several additional features: a large multiple cloning site (MCS) for traditional cloning, the resistance marker to ampicillin, and C-terminal His•Tag® sequence (with 10 histidine residues). Vectors featuring the 3C protease site also encode a C-terminal thrombin (Tb) protease site for removal of the C-terminal His•Tag sequence. With options for "gentle elution" tags at both the N-terminus (Strep•Tag II) and C-terminus (His•Tag), these vectors are ideal for dual purification strategies designed to isolate full-length, highly purified fusion proteins.

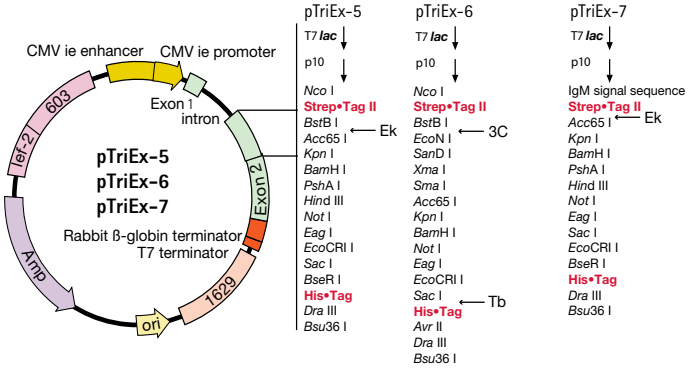
The pIEx-10 and pTriEx-7 also feature the mouse IgM signal sequence for secretion of fusion proteins from insect cells (pIEx-10), or both insect and mammalian cells, and export to the periplasm in *E. coli* cells (pTriEx-7).

Features:

- Vectors for expression in bacterial (pET), insect (InsectDirect), and multi (pTriEx) systems
- Small-8 amino acid Strep•Tag II fusion tag
- Two Ligation-independent Cloning (LIC) options: Ek and 3C

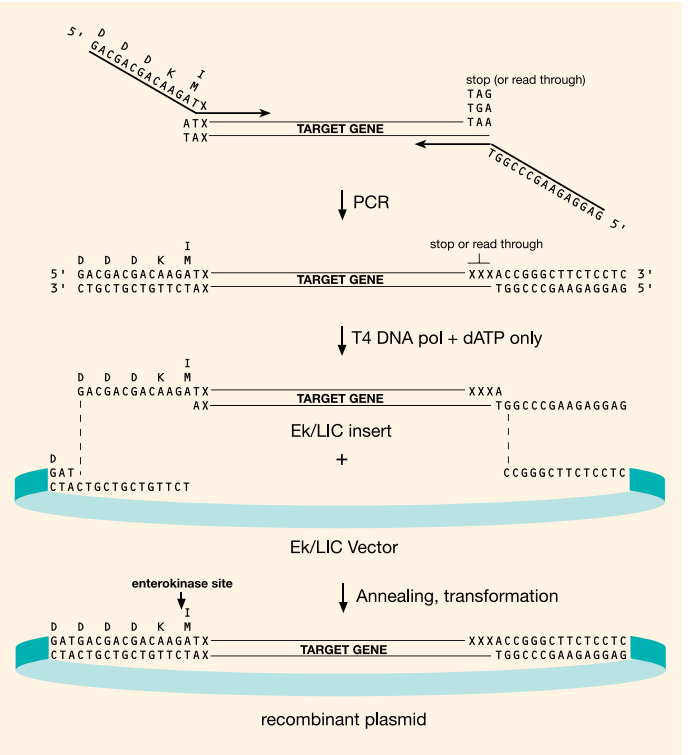






Ligation-independent Cloning

Strep•Tag® II vectors are also available in the convenient Ligation-independent Cloning (LIC) format. The LIC method enables high-efficiency directional cloning without restriction enzyme digestion or ligation reactions. pET-51b, pIEx™-8, pIEx-10, pTriEx™-5, and pTriEx-7 are all members of the Radiance™ Cloning System based on Ek/LIC cloning, while pET-52b, pIEx-9 and pTriEx-6 are available in a 3C/LIC format. The single-stranded overhangs present in the 3C/LIC system complete the HRV 3C protease site coding sequence upstream of the insert and initiate the thrombin protease site coding sequence downstream of the insert. This new configuration allows the removal of the N-terminal and/or C-Terminal fusion tags by the appropriate protease, if desired.



Schematic of Radiance™ Ek/LIC cloning strategy

Vector Characteristics Table

	Host and Promoter System				N-Terminal Features				C-Terminal Features		Antibiotic Resistance
Vector	*E. coli* T7*lac* promoter	Insect cells (Plasmid) hr5 enhancer, ie1 promoter	Insect cells (Baculovirus) p10 promoter	Mammalian CMV ie enhancer and promoter	Secretion signal	Strep•Tag II	Ek/LIC or Ek site	3C/LIC or 3C site	Tb site	10x His•Tag®	Amp
pET-51b(+)	X					X	X			X	X
pET-52b(+)	X					X		X	X	X	X
pIEx-8		X				X	X			X	X
pIEx-9		X				X		X	X	X	X
pIEx-10		X			X	X	X			X	X
pTriEx-5	X		X	X		X	X			X	X
pTriEx-6	X		X	X		X		X	X	X	X
pTriEx-7	X		X	X	X	X	X			X	X

3C: HRV 3C; Ek: enterokinase; Tb: thrombin

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Protein Purification

NEW Strep•Tag® II Purification

Strep•Tactin® resins offer rapid one-step affinity purification of proteins containing the Strep•Tag® II fusion tag while preserving the bioactivity of the protein. After Strep•Tag II recombinant proteins are expressed, cells are lysed, and the lysate is added to a column or cartridge containing immobilized Strep•Tactin affinity resin. The column/cartridge is washed several times to remove nonspecifically bound proteins, and bound Strep•Tag proteins are gently eluted with 2.5 mM desthiobiotin. Desthiobiotin is an analog of biotin that competes for the Strep•Tag II binding site and specifically releases the Strep•Tag fusion proteins.

- Rapid one-step affinity purification
- >95% purity of bioactive fusion proteins
- Compatible with a broad range of detergents, chelators, and salts
- Suitable for isolation of protein complexes
- Regenerate column 3-5 times
- Visual confirmation of regeneration (see page 8)

Two different resin supports are available. The Strep•Tactin Superflow™ Agarose is a cross-linked agarose derivatized with Strep•Tactin protein that can be used for gravity flow as well as for low pressure and FPLC chromatography. The Strep•Tactin MacroPrep® Resin is a polymethacrylate resin suitable for gravity flow and all low pressure chromatography applications. This resin exhibits non-specific binding properties that differ from the Strep•Tactin Superflow Agarose. Therefore, if non-specific protein contamination is a problem with a particular expression host or recombinant protein, using the alternative resin may improve the purification. Strep•Tactin has a binding capacity of 50–100 nmol, depending upon the size of the recombinant protein, and a dissociation constant (K_d) of 1 µM. Both types of resins are available in a variety of convenient formats: pre-packed columns for gravity flow, cartridges for use with low pressure chromotography (LPLC) or syringes, 96-well plates, spin columns, and resin slurries. In many of these formats, the resin can be reused 3-5 times without a loss in binding capacity (see the Matrix Selection Guide on p. 9 for details).

Strep•Tactin Superflow and MacroPrep Resin slurries are available and offer the flexibility of adjusting the affinity purification scale to meet specific experimental needs. However, because Strep•Tag/Strep•Tactin purification has been optimized for column purification, batch methods are not recommended.

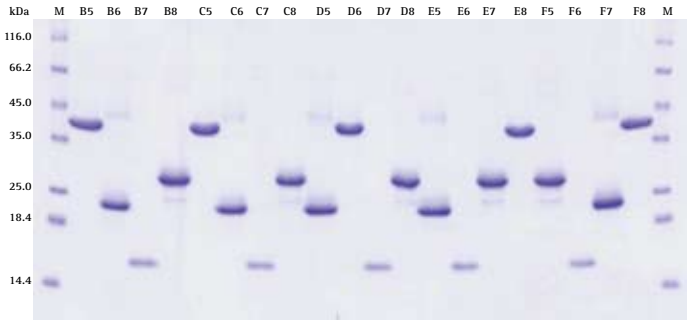
For added value and convenience, a Strep•Tactin Buffer Kit, consisting of a set of pre-tested buffers designed for optimal use with Strep•Tactin resins, is also available.

Product	Size	Cat. No.	Price
Introductory Strep•Tag® II Kit, pET-51b*	1 kit	71615-3	
Introductory Strep•Tag II Kit, pET-52b*	1 kit	71616-3	
Components: • 10 µg pET-51b or pET-52b DNA • 1 Strep•Tactin® Superflow™ Column, 1 ml • 120 ml 1X Strep•Tactin Wash Buffer • 25 ml 1X Strep•Tactin Elution Buffer • 120 ml 1X Strep•Tactin Regeneration Buffer • 20 µl Strep•Tag II Antibody HRP Conjugate • 0.2 ml Induction Control			
Strep•Tactin® Superflow™ Agarose*	2 ml 10 ml	71592-3 71592-4	
Strep•Tactin Superflow Column, 0.2 ml*	5 columns	71594-3	
Strep•Tactin Superflow Column, 1 ml*	5 columns	71593-3	
Strep•Tactin Superflow Cartridge, 1 ml*	5 cartridges	71595-3	
Strep•Tactin Superflow Cartridge, 5 ml*	1 cartridges 5 cartridges	71596-3 71596-4	
Strep•Tactin MacroPrep® Resin*	2 ml 10 ml	71597-3 71597-4	
Strep•Tactin MacroPrep Cartridge, 1 ml*	5 cartridges	71598-3	
Strep•Tactin Buffer Kit*	1 kit	71613-3	
Components: • 100 ml 10X Strep•Tactin Wash Buffer • 25 ml 10X Strep•Tactin Elution Buffer • 100 ml 10X Strep•Tactin Regeneration Buffer			
10X Strep•Tactin Wash Buffer*	100 ml	71603-3	
10X Strep•Tactin Elution Buffer*	25 ml	71604-3	
10X Strep•Tactin Regeneration Buffer*	100 ml	71612-3	
D-Desthiobiotin*	1 g	71610-3	

*Strep•Tactin resins, Strep•Tactin buffers, and Strep•Tag II Antibodies are manufactured by IBA GmbH.

Strep•Tactin® HT96™ Purification Kit

The Strep•Tactin® HT96™ Purification Kit is designed for Strep•Tag® II fusion protein purification in a 96-well format. The Strep•Tactin HT96 Plate contains predispensed Strep•Tactin resin that simply requires rehydration and equilibration before use. The kit purifies up to 100 µg/well of Strep•Tag fusion protein. The kit is compatible with standard vacuum manifolds for manual sample processing and with robotic liquid handling systems. The kit contains one each of Strep•Tactin HT96 Purification Plate, Prefilter Plate, Wash Plate, Receiver Plate, and buffers.



Strep•Tag II constructs containing either GAPDH (37.5 kDa), GFP (28.1 kDa), GSHH (23.5 kDa), or azurin (15.1 kDa) coding sequences were cloned into expression vectors in *E. coli* and plated on selective medium. 96 colonies were randomly picked, grown in 5 ml cultures, and the cultures were induced. Subsequently, the cells were harvested, lysed, and Strep•Tag fusion proteins were purified using the Strep•Tactin HT96 Purification Kit. 8 µl from each of 20 wells was analyzed by SDS-PAGE.

Product	Size	Cat. No.	Price
Strep•Tactin® HT96™ Purification Kit*	1 plate	71605-3	
Components: • 1 Strep•Tactin HT96 Purification Plate • 1 Strep•Tactin HT96 Prefilter Plate • 1 Strep•Tactin HT96 Wash Plate • 1 Strep•Tactin HT96 Receiver Plate • 50 ml 10X Strep•Tactin Wash Buffer • 50 ml 1X Strep•Tactin Elution Buffer, with Desthiobiotin			
Strep•Tactin SpinPrep™ Kit*	25 rxn	71608-3	
Components: • 25 Strep•Tactin SpinPrep Columns and Collection Tubes • 120 ml 1X Strep•Tactin Wash Buffer • 25 ml 1X Strep•Tactin Elution Buffer, with Biotin			
M6 Adaptor Set (for FPLC)*	1 set	71586-3	
1/4-28 Adaptor Set (for FPLC)*	1 set	71587-3	
10-32 Adaptor Set (for HPLC and Äkta)*	1 set	71588-3	
1/16 Inch Adaptor Set (for peristaltic pump tubing)*	1 set	71589-3	

*Manufactured by IBA GmbH.

Strep•Tactin SpinPrep™ Kit

The Strep•Tactin SpinPrep™ Kit offers a fast and easy-to-handle method for purifying Strep•Tag II fusion proteins using small spin columns containing Strep•Tactin resin. Each column purifies up to 150 µg of Strep•Tag II fusion protein. The kit is supplied with 25 Strep•Tactin SpinPrep Columns, collection tubes, and optimized wash and elute buffers.

Strep•Tactin Cartridge Adaptor Sets

The adaptors for the Strep•Tactin Superflow™ and MacroPrep® Cartridges provide compatibility with liquid chromatography systems and FPLC workstations. The 10-32 Adaptor Set is for use with the GE Healthcare Äkta system. The M6 Adaptor Set is for use with GE Healthcare FPLC systems, other than Äkta systems. The 1/4-28 Adaptor Set is for use with other low pressure chromatography systems. The 1/16 Inch Adaptor Set is for use with peristaltic pumps.

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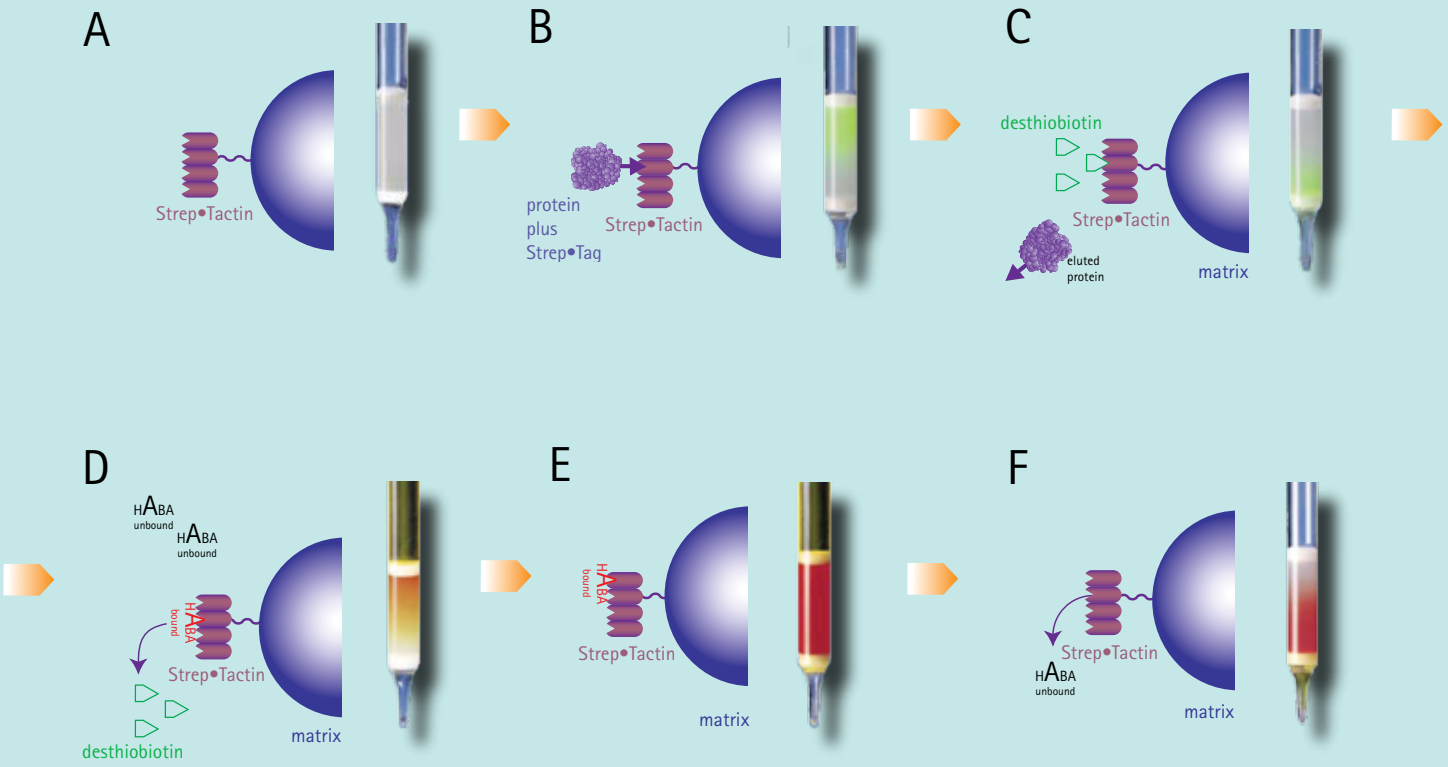
The complete cycle of protein purification and column regeneration



The purification of a Strep•Tag® II fusion protein and regeneration of the Strep•Tactin® resin is straightforward and easy. After equilibration of a Strep•Tactin column with the desired binding buffer, (i.e., 1X Strep•Tactin Wash Buffer, 1X PBS, or another physiological buffer), the column is ready for use (Figure A). The cell lysate, in this case containing a Strep•Tag II/GFP fusion protein, is added to the column (Figure B) and the column is washed several times with 1X Strep•Tactin Wash Buffer to remove nonspecific proteins. Bound Strep•Tag II/GFP fusion protein is gently eluted by the addition of 2.5 mM desthiobiotin, which specifically competes for the Strep•Tactin binding pocket (Figure C). To regenerate the column, an excess of a yellow azo dye, hydroxyl-azophenyl-benzoic acid (HABA), which displaces the desthiobiotin from the binding pocket on the Strep•Tactin, is added to the column (Figure D). When HABA binds to the site, it changes color from yellow to red, so complete regeneration of the column is conveniently indicated by the red color (Figure E). HABA is then removed by adding 1X Strep•Tactin Wash Buffer (Figure F) and the column is ready for reuse. Strep•Tactin resins can be conveniently regenerated and reused 3-5 times without a loss in performance.

 For more information or to place an order, contact your local office (see back cover).

Strep•Tactin® Matrix Selection Guide

Product	Form	Capacity	Features	Applications
Strep•Tactin® Superflow™ Agarose*	Superflow 6 agarose	50-100 nmol/ml	Reusable 3-5 times; Compatible with up to 1 M urea or guanidine, 2% Triton® X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Small to production scale FPLC or gravity-flow column
Strep•Tactin Superflow Column*, 0.2 ml	Superflow 6 agarose, pre-packed column	10-20 nmol	Reusable 3-5 times; Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Convenient purification Gravity-flow column
Strep•Tactin Superflow Column*, 1 ml	Superflow 6 agarose, pre-packed column	50-100 nmol	Reusable 3-5 times; Luer fitting on one end; Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Convenient purification Gravity-flow column
Strep•Tactin Superflow Cartridge*, 1 ml	Superflow 6 agarose, pre-packed cartridge	50-100 nmol	Reusable 3-5 times; Compatible with Luer adaptors; Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Syringe processing FPLC workstation purification Rapid purification
Strep•Tactin Superflow Cartridge*, 5 ml	Superflow 6 agarose, pre-packed cartridge	250-500 nmol	Reusable 3-5 times; Compatible with Luer adaptors; Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Syringe processing FPLC workstation Rapid purification
Strep•Tactin MacroPrep® Resin*	Polymethacrylate	50-100 nmol/ml	Reusable 3-5 times; 50 µm particle size; High flow rates and pressures; Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Small to production scale FPLC or gravity-flow column
Strep•Tactin MacroPrep Cartridge*	Polymethacrylate, pre-packed cartridge	50-100 nmol	Reusable 3-5 times; Compatible with Luer adaptors; 50-µm particle size; High flow rates and pressures; Compatible with up to 1 M urea or guanidine, and 50 mM DTT or 2-ME.	Syringe processing FPLC/HPLC workstation Rapid purification
Strep•Tactin HT96™ Purification Plate*	Superflow 6 agarose	100 µg/well	Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	High-throughput robotic processing Vacuum manifold Parallel processing
Strep•Tactin SpinPrep™ Column*	Agarose, pre-packed column	150 µg	Compatible with up to 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME.	Rapid convenient purification Parallel processing Ready-to-use spin column

Note: The Strep•Tag®/Strep•Tactin purification system is designed for column purification and is not recommended for use in batch purification.
Abbreviations: 2-ME: 2-mercaptoethanol; DTT: dithiothreitol

Buffer Compatibility Guide

Reagent	Concentration
Reduction Agents	
DTT	50 mM
2-mercaptoethanol	50 mM
Non-ionic Detergents	
C_8E_4; Octyltetraoxyethylene	0.88%
$C_{10}E_5$; Decylpentaoxyethylene	0.12%
$C_{12}E_8$; Octaethyleneglycol Mono-*n*-dodecyl Ether	0.005%
$C_{12}E_9$; Dodecyl nonaoxyethylene (Thesit)	0.023%
Decyl-β-D-maltoside	0.35%
n-dodecyl-β-D-maltoside	0.007%
n-nonyl-β-D-glucopyranoside	0.2%
n-octyl-β-D-glucopyranoside	2.34%
Triton® X-100	2%
TWEEN® 20	2%
BugBuster® Extraction Reagent	1X
Ionic Detergents	
n-lauryl-sarcosine	2%
8-HESO; *n*-octyl-2-hydroxy-ethylsulfoxide	1%
SDS; Sodium-*n*-dodecyl sulfate	0.1%

Reagent	Concentration
Zwitterionic Detergents	
CHAPS	0.1%
DDAO; *n*-decyl-N,N-dimethylamine-N-oxide	0.034%
LDAO; *n*-dodecyl-N,N-dimethylamine-N-oxide	0.13%
Others	
Ammonium sulfate $(NH_4)_2SO_4$	2 M
$CaCl_2$	1 M
EDTA	50 mM
Ethanol	10%
Guanidine	1 M
Glycerol	25%
Imidazole	250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	1 M

Note: These reagents have been successfully tested for purification of GAPDH-Strep•Tag II with concentrations up to those mentioned. Since binding depends on the steric accessibility of Strep•Tag II in the context of the particular protein, concentrations may deviate from the given value for different proteins.

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Isolating protein complexes from mammalian cells

The identification of protein:protein interaction complexes is important to understanding metabolic and disease mechanisms. To identify complexes, a method must be stringent enough to differentiate binding to the protein of interest versus nonspecific binding, and yet be gentle enough to not dissociate the complex. The One•STrEP Protein Interaction Kit provides an extremely fast and efficient single-step method to isolate intact protein complexes from mammalian cells by using the One-STrEP•Tag fusion tag, a fusion tag that consists of two Strep•Tag® II fusion tags separated by a spacer. Unlike other systems that require tedious optimization or multiple purification steps, this system takes advantage of the highly selective Strep•Tactin® Superflow™ Column for fast protein purification, under physiological conditions, with low background. With the One•STrEP Protein Interaction Kit, you can isolate weakly and/or transiently associated protein complexes. This kit provides the pEXPR-IBA103 Mammalian Expression Vector, which is designed for a C-terminal One-STrEP•Tag fusion to the bait protein. The vector features a CMV promoter for expression in a variety of mammalian cells, and a neomycin resistance marker to allow for selection of stable cell lines. Also included are Strep•Tactin Superflow columns, and buffers for the isolation of protein complexes at different scales, a Strep•Tag II Antibody HRP Conjugate for detection, and a positive control.

Product	Size	Cat. No.	Price
One•STrEP Protein Interaction Kit*	1 kit	71624-3	

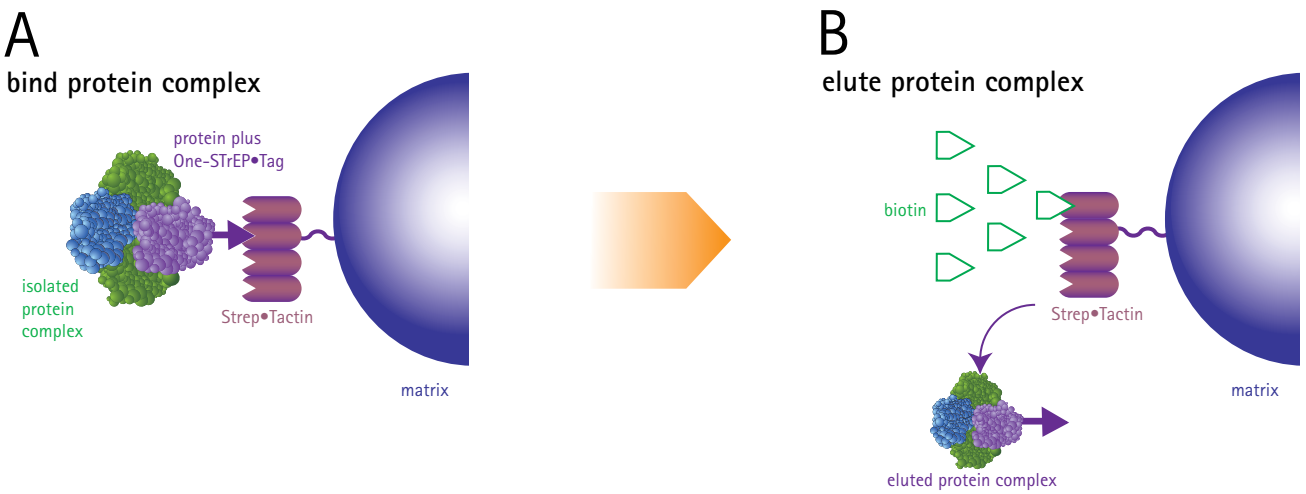
Components:

• 5 µg	pEXPR-IBA 103 Mammalian Expression Vector
• 5	Strep•Tactin® Superflow™ Column, 1ml
• 5	Strep•Tactin Superflow Column, 0.2 ml
• 2 × 75 ml	1X Strep•Tactin Lysis Buffer
• 120 ml	1X Strep•Tactin Wash Buffer
• 25 ml	1X Strep•Tactin Elution Buffer Buffer with Biotin
• 2 × 20 µl	Strep•Tag® II Antibody, HRP Conjugate
• 50 µl	PP2Ac Goat Polyclonal Antibody
• 5 µg	One•STrEP Control Vector, lyophilized

*Manufactured by IBA GmbH.

Features:

- Isolates intact protein complexes in a single step
- Results in minimal background
- Preserves transient and weak protein interactions



Protein Detection

Detecting Strep•Tag® II Fusion Proteins

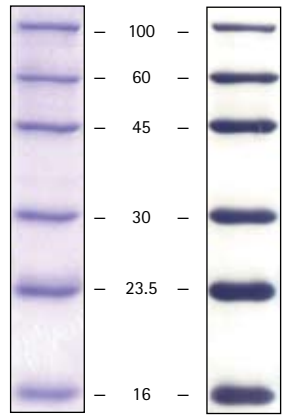
In addition to protein purification, the Strep•Tag® II peptide can be used to detect Strep•Tag II fusion proteins. A mouse monoclonal antibody (IgG_1) is available that specifically recognize the 8-amino acid Strep•Tag II sequence. The highly specific Strep•Tag II Monoclonal Antibody and Strep•Tag II Antibody HRP Conjugate exhibit no cross reactivity with other proteins in bacterial, insect, or mammalian extracts, and can detect as little as 5 ng Strep•Tag II fusion protein. The antibodies are suitable for Western and dot blots.

For accurate size determination of the Strep•Tag II fusion proteins, Strep•Tag II Perfect Protein™ Markers, ranging from 16 to 100 kDa, provide precise protein size references on SDS-polyacrylamide gels after staining with RAPIDstain™ Reagent or Coomassie blue. Because each marker protein contains the Strep•Tag II sequence, the markers can be detected by the Strep•Tag II Monoclonal Antibody or Strep•Tag II Antibody HRP Conjugate in Western blots. Markers are provided lyophilized and are supplied with Strep•Tag II Marker Reconstitution Buffer, which also serves as the loading buffer.

Product	Size	Cat. No.	Price
Strep•Tag® II Monoclonal Antibody*	100 µg	71590-3	
Strep•Tag II Antibody HRP Conjugate*	75 µl	71591-3	
Strep•Tag II Perfect Protein™ Markers*	100 lanes	71614-3	
Components: • 1 vial Strep•Tag II Perfect Protein Markers, Lyophilized • 500 µl Strep•Tag II Marker Reconstitution Buffer			
Goat Anti-Mouse IgG AP Conjugate (H + L)	40 µl	69266-3	
Goat Anti-Mouse IgG HRP Conjugate (H + L)	40 µl	71045-3	

*Manufactured by IBA GmbH.

Coomassie staining Chemiluminescent detection



Strep•Tag II Perfect Protein Markers (5 µl) were run in duplicate on SDS-PAGE. One lane of the gel was stained by Coomassie blue while the other lane was transfered to nitrocellulose and analyzed by Western blot. The blot was incubated with the Strep•Tag II Monoclonal Antibody, followed by a goat anti-mouse IgG HRP conjugate. The markers were detected by chemiluminescence.

Development Substrates and Stains

AP and HRP Blot Development Substrates

Quality substrates used for signal development are optimized to achieve high sensitivity and low background in Western and dot blots. The AP Detection Reagent Kit provides sensitive chromogenic detection of alkaline phosphatase conjugates. For very high sensitivity, chemiluminescent detection is recommended. Both CDP-*Star*® AP Substrate and SuperSignal® HRP Substrate enable subnanogram sensitivity in a convenient ready-to-use format.

RAPIDstain™ Reagent

RAPIDstain™ Reagent is an ultra-sensitive Coomassie-based reagent for staining polyacrylamide gels. No mixing or preparation, no fixation, and no destaining are necessary. RAPIDstain Reagent stains only protein, within 5-10 minutes, and leaves a crystal-clear background providing high resolution. Sensitivity: 4-8 ng BSA.

Product	Size	Cat. No.	Price
AP Detection Reagent Kit (NBT, BCIP, 20X AP Buffer)	1 ea 5 ea	69264-3 69264-4	
CDP-*Star*® AP Substrate	40 ml	69086-3	
SuperSignal® HRP Substrate	2 × 25 ml	69059-3	
RAPIDstain™ Reagent	1 L	553215	

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FAQ about Strep•Tag® II / Strep•Tactin® Affinity Purification Products

What is the principle of Strep•Tag technology?

The Strep•Tag® II purification system is based on the highly selective and easily controlled interaction between the Strep•Tag II peptide and Strep•Tactin®, a specifically engineered streptavidin. The tagged protein binds to immobilized Strep•Tactin in physiological buffers, like PBS, in combination with a wide range of additives. After a brief wash, purified, recombinant protein is gently eluted by adding 2.5 mM desthiobiotin. Desthiobiotin is a reversibly binding stable analog of biotin—the natural ligand of streptavidin.

What is the size of Strep•Tag II?

Strep•Tag II peptide is eight amino acids (TrpSerHisProGlnPheGluLys) and has a molecular weight of 1 kDa.

What is the binding affinity of the Strep•Tag II for Strep•Tactin?

K_d = 1 µM

What is the binding capacity of the Strep•Tactin resins?

For both the Strep•Tactin Superflow™ Agarose and the Strep•Tactin MacroPrep® resin, the binding capacity is 50-100 nmol/ml of fusion protein, depending on the size of the protein.

What degree of purity can be expected?

Over 95%. However, impurities resulting from nonspecific interactions with the recombinant protein itself could lead to lower purity. To reduce contaminants, additives such as reducing agents, salts, or mild detergents can be used in the bind, wash, and elution buffers.

Does Strep•Tag II bind avidin?

No. Therefore, avidin can be used to block naturally occurring biotin in cell lysates and from culture medium.

For proteins expressed in the cytoplasm, is the presence of biotinylated proteins in the host organism a problem?

No. Generally, the amount of biotinylated proteins in the cytoplasm is very low and does not lead to significant inactivation of the column. In an *E. coli* extract derived from a 1 L culture with OD_{550} = 1, the total biotin content is approximately 1 nmol; column capacity is 350 nmol/ml. Even the biotinylated *E. coli* biotin carboxyl carrier protein (BCCP) has a relatively low intracellular concentration and usually does not interfere with purification. However, to avoid binding biotin irreversibly to Strep•Tactin resin, add avidin to the cell lysate before chromatography (20 µg/L for an *E. coli* culture at OD_{550} = 1).

Is the protein complexed with desthiobiotin when it is eluted from the column?

No. Desthiobiotin does not complex or interfere with the protein or general protein assays, and can be removed by gel filtration or dialysis.

Is it possible to detect and isolate protein–protein interactions using Strep•Tag II technology?

Yes. The One•STrEP Protein Interaction Kit (Cat. No. 71624) was specifically designed to isolate intact protein complexes.

Is there a convenient method for parallel purification of different Strep•Tag II proteins?

Yes. The Strep•Tactin HT96™ Purification Kit (Cat. No. 71605) was designed for automated, high-throughput purification of Strep•Tag II proteins (up to 100 µg Strep•Tag II protein per well). The plates are pre-loaded with Strep•Tactin affinity resin and can be used with standard vacuum manifolds for manual sample processing or with robotic sample processing systems.

Can the Strep•Tag II be removed?

Yes. The Novagen® vectors contain either an enterokinase (Ek) or HRV 3C cleavage site. However, due to the small size and chemically inert nature of Strep•Tag II, it generally does not interfere with the folding or bioactivity of the recombinant protein and therefore does not need to be removed.

Can detergents or other buffer systems be used?

Yes. As long as the pH remains above pH 7.5, high salt, reducing reagents, chelating reagents, and detergents can be used with Strep•Tactin. The resin is also compatible with BugBuster® Protein Extraction Reagent (Cat. No. 70584) and high protein yields have been achieved using BugBuster Master Mix (Cat. No. 71456). See the table on p. 9 for a list of reagents that have been successfully tested.

How is Strep•Tactin resin regenerated?

The matrix is regenerated with an azo dye, hydroxyl-azophenyl-benzoic acid (HABA), which, when applied in excess, displaces desthiobiotin. The dye is yellow in solution and shifts to red when bound by Strep•Tactin, allowing visual control of the regeneration process and the functional status of the column. The resin can be regenerated 3-5 times.

Supporting tools for protein expression and purification

Bacterial expression

Competent Cells: Cloning and Expression Hosts

Novagen competent cells encompass the widest selection available for protein expression and offer fundamental strains for cloning applications. The NovaBlue strain is an excellent choice for routine cloning due to its high transformation efficiency and mutations in *recA* and *endA*, which allow high yields of high quality plasmid DNA. For protein expression, available hosts provide stringent control over basal expression levels (pLysS strains), enable disulfide bond formation in the cytoplasm (Origami™ 2 and Rosetta-gami™ 2 strains), and alleviate codon usage incompatibilities (Rosetta™ 2 and Rosetta-gami 2 strains). BL21 is an ideal starting strain for recombinant protein expression. BL21 and its derivatives (i.e., Rosetta 2) are deficient in both *lon* and *ompT* proteases. The Singles™ competent cell format is designed for ultimate convenience and reliability in plasmid transformation. Chemically competent cells are provided in 50-µl volumes that eliminate the need to aliquot, freeze/thaw, or waste partially used vials, saving time and providing increased performance.

Product		Size	Cat. No.	Price
Cloning Strain	NovaBlue Singles™ Competent Cells	11 rxn 22 rxn	70181-3 70181-4	
T7 Expression Strains*	BL21(DE3) Singles Competent Cells	11 rxn 22 rxn	70235-3 70235-4	
	BL21(DE3) pLysS Singles Competent Cells	11 rxn 22 rxn	70236-3 70236-4	
	Rosetta™ 2(DE3) Singles Competent Cells	11 rxn 22 rxn	71400-3 71400-4	
	Origami™ 2(DE3) Singles Competent Cells	11 rxn 22 rxn	71408-3 71408-4	
	Rosetta-gami™ 2(DE3) Competent Cells	0.4 ml** 1 ml**	71351-3 71351-4	

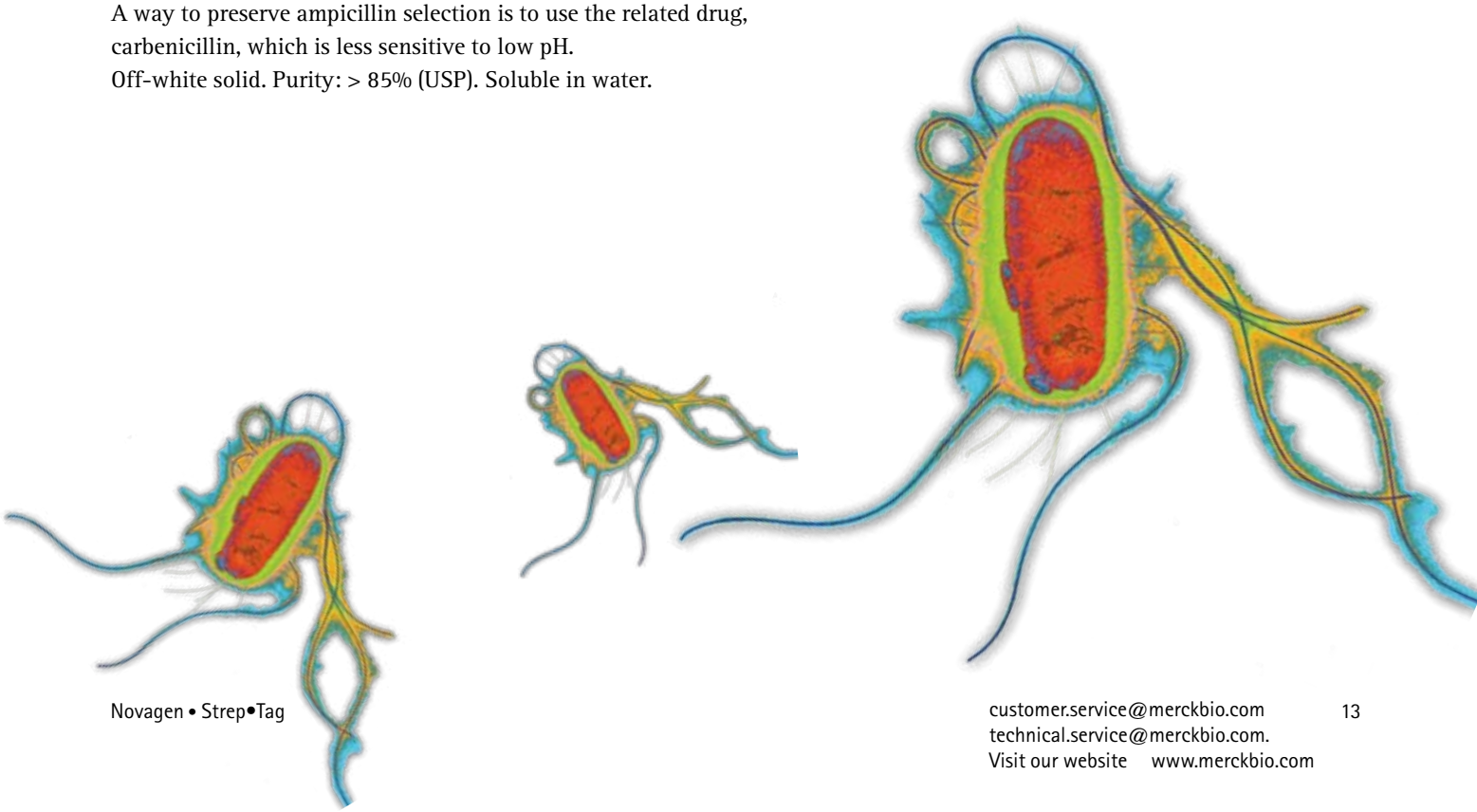
* For a complete description and listing of Novagen expression host strains, visit www.novagen.com/compcells

** Provided as a 0.2-ml format: 0.4 ml is suficient for 20 rxn and 1 ml for 50 rxn.

Carbenicillin

Carbenicillin is an antibiotic that interferes with the terminal reaction in bacterial wall synthesis. It is recommended for use in place of ampicillin to maintain the selective marker *bla* (β-lactamase, or ampicillin resistance). Ampicillin selection tends to be lost in cultures as the drug is degraded by the secreted β-lactamase enzyme and by the drop in pH that usually accompanies bacterial fermentation. A way to preserve ampicillin selection is to use the related drug, carbenicillin, which is less sensitive to low pH.
Off-white solid. Purity: > 85% (USP). Soluble in water.

Product	Size	Cat. No.	Price
Carbenicillin	69101-3	5 g	



Overnight Express™ Autoinduction Media

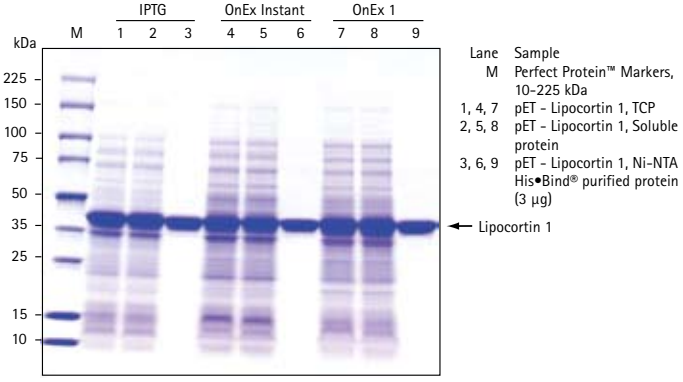
With the introduction of Overnight Express™ Autoinduction media, proteins can be expressed effortlessly and at significantly higher yields than with IPTG induction. Overnight Express Autoinduction system gives spontaneous induction of recombinant protein expression following a period of cell growth, without monitoring cell densities or adding IPTG. The method of growth and induction used in this system relies on media components that are metabolized differentially to promote high-density growth and automatic induction of *lac* promoter driven expression. Autoinduction with Overnight Express is scalable and convenient: similar protein expression efficiencies are achieved in a 50-ml flask or a 15-L fermenter.

Features

- Available as Overnight Express Autoinduction System 1 (added to glucose-free complete media), Overnight Express Autoinduction System 2 (chemically defined medium), and Overnight Express Instant TB Medium (granulated complete medium)
- For Overnight Express Instant TB Medium, just add water and glycerol, and autoclave or microwave to sterilize
- Produces high cell densities and protein expression levels
- No need to monitor cell growth rate or add inducer
- Ideal for pET Expression System or other IPTG-inducible bacterial systems
- Overnight Express Autoinduction System 2 enables efficient selenomethionine labeling

Product	Size	Cat. No.	Price
Overnight Express™ Instant TB Medium	1 EasyPak	71491-3	
	5 EasyPaks	71491-4	
	1 kg	71491-5	
Overnight Express Autoinduction System 1	1 kit*	71300-3	
	1 kit†	71300-4	
Overnight Express Autoinduction System 2	1 kit*	71366-3	
	1 kit†	71366-4	

* 71300-3 and 71366-3 include enough reagents to induce 1 L.
† 71300-4 and 71366-4 include enough reagents to induce 5 L.



Analysis of crude and purified samples obtained from 15-L fermentations
Fermentations were inoculated with a 500-ml starter culture. Final culture volume was 15 L in the media described here. Fermentation in Overnight Express Instant TB was accomplished by a 17.5-h growth at 30°C to an OD_{600} of 10.1. Fermentation in TB + System 1 was accomplished by a 16-h growth at 30°C to an OD_{600} of 9.9. IPTG induction during fermentation was accomplished by a 3.5-h growth at 37°C, decreasing the temperature to 30°C, and adding IPTG to a final concentration of 1.0 mM at an OD_{600} of 1.3. Cells were harvested after a 4-h IPTG induction at an OD_{600} of 5.5. Equivalent cell masses of total cell protein (TCP) and soluble protein fractions from cellular extracts were analyzed by SDS-PAGE and stained with Coomassie blue.
OnEx: Overnight Express

Protein Extraction

BugBuster® Master Mix

BugBuster® Master Mix combines BugBuster Protein Extraction Reagent with Benzonase® Nuclease and rLysozyme™ Solution in one convenient reagent. BugBuster Protein Extraction Reagent is ideal for extracting active soluble proteins from *E. coli* without sonication or other mechanical disruption methods. BugBuster Master Mix significantly increases protein extraction efficiency and facilitates downstream processing of extracts from both Gram-negative and Gram-positive bacteria. Two package sizes are available and provide sufficient reagents for protein extraction from 20 g or 100 g cell paste.

Product	Size	Cat. No.	Price
BugBuster Master® Mix	100 ml	71456-3	
	500 ml	71456-4	

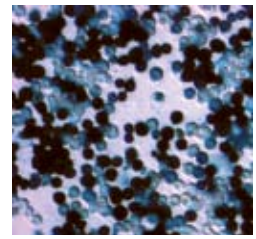
Insect Expression

Insect GeneJuice® Transfection Reagent

Insect GeneJuice® Transfection Reagent is a proprietary liposome formulation optimized for maximal transfection efficiency of insect cells. The reagent features extremely low cytotoxicity and can be used for both transient and stable transfections in serum-containing or serum-free media. Insect GeneJuice Transfection Reagent is ideal for large-scale protein expression when using vectors such as the pIEx™ and pBiEx™ vectors for transfection of Sf9 and other insect cells. The reagent is also suitable for conventional cotransfection of BacVector® Triple Cut Virus DNA or BacMagic™ DNA and pBAC™ or pTriEx™ transfer plasmids into Sf9 insect cells to construct recombinant baculoviruses. Insect GeneJuice Transfection Reagent is provided as a 2 mg/ml suspension in 20 mM MES, 150 mM NaCl, pH 6.2 buffer. One ml is sufficient for 12 or 125 transfections in 10-ml suspension culture flasks or 35-mm plates, respectively.

Product	Size	Cat. No.	Price
Insect GeneJuice® Transfection Reagent	0.3 ml	71259-3	
	1 ml	71259-4	
	10 × 1 ml	71259-5	

BULK



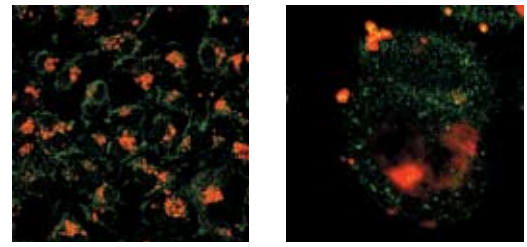
Sf9 cells transfected with pIEx-1/β-gal using Insect GeneJuice Transfection Reagent
Transfected cells were stained for β-gal activity using the BetaBlue™ Staining Kit.

Mammalian Expression

GeneJuice® Transfection Reagent

GeneJuice® Transfection Reagent is a proprietary non-liposomal formulation optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity. This reagent is a superior alternative to lipid-based reagents, as well as traditional techniques including calcium phosphate coprecipitation, electroporation, and complex formation with DEAE-dextran. GeneJuice Transfection Reagent provides excellent performance for both stable and transient transfection of mammalian cells. The 1-ml size provides enough reagent to perform up to 500 transfections in standard 35-ml plates. GeneJuice Reagent is supplied as a ready-to-use sterile solution.

Product	Size	Cat. No.	Price
GeneJuice® Transfection Reagent	0.3 ml	70967-5	
	1 ml	70967-3	
	5 x 1 ml	70967-6	
	10 × 1 ml	70967-4	



GeneJuice transfection of COS-7 and HeLa cells with rhodamine-labeled DNA
Cells grown on polylysine-coated coverslips were transfected with rhodamine-labeled pTriEx™-2 DNA using GeneJuice. Twenty-four hours after transfection, the cells were washed in PBS, fixed in 4% formalin, and washed again in PBS. Coverslips were mounted on glass slides and sealed for confocal microscopy. The transfected DNA is seen in red. Unfiltered reflected light from the 533 nm laser was collected to image the cell boundaries.

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