
December 2021

TAGZyme Handbook

For exoproteolytic cleavage of
N-terminal His tags

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Kit Contents

TAGzyme pQE Vector Catalog no.	32932
TAGzyme pQE-2 Vector DNA	25 µg

TAGzyme DAPase™ Enzyme (2.5 U) – for processing of approx. 50 mg of tagged protein Catalog no.	34362
DAPase Enzyme (10 U/ml)	2.5 units (250 µl)
Cysteamine-HCl (20 mM)	1000 µl
DAPase Enzyme Product Sheet	1

TAGzyme DAPase Enzyme (50 U)* – for processing of approx. 1 g of tagged protein Catalog no.	34366
DAPase Enzyme (10 U/ml)	50 units (5 µl)
Cysteamine-HCl (20 mM)	25 µl
DAPase Enzyme Product Sheet	1

* Bulk enzyme quantities are customized products; delivery may take up to 6 weeks. Enzymes are also available in GMP-grade. Please inquire.

Storage

DAPase and TAGZyme pQE-2 Vector DNA should be stored at -30 to -15°C .

See Appendix A for enzyme stability information.

Intended Use

The TAGZyme Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of TAGZyme Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The TAGZyme System is an efficient and specific solution for the complete removal of small N-terminal His tags and other amino acid tags by the use of exopeptidases. The method is based on the use of dipeptidyl aminopeptidase I (DAPase Enzyme). This recombinant enzyme contains a C-terminal His tag and can therefore be bound to Ni-NTA matrices. This allows their removal from the reaction solution by Immobilized-Metal Affinity Chromatography (IMAC). This feature has been utilized in the design of a simple process consisting of aminopeptidase cleavage followed by subtractive IMAC. The system can form the backbone of a combined TAGZyme–IMAC strategy for the efficient production of highly purified and homogeneous recombinant proteins (see Figure 1). As the TAGZyme enzyme carries a His tag at their C-terminus, the tag is not digested by the N-terminal exopeptidase DAPase Enzyme.

The use of exopeptidases in the TAGZyme System eliminates the risk of endoproteolytic cleavage within the body of the protein and the rapid rate of reaction means that sensitive proteins can be processed even at 4°C in a matter of hours.

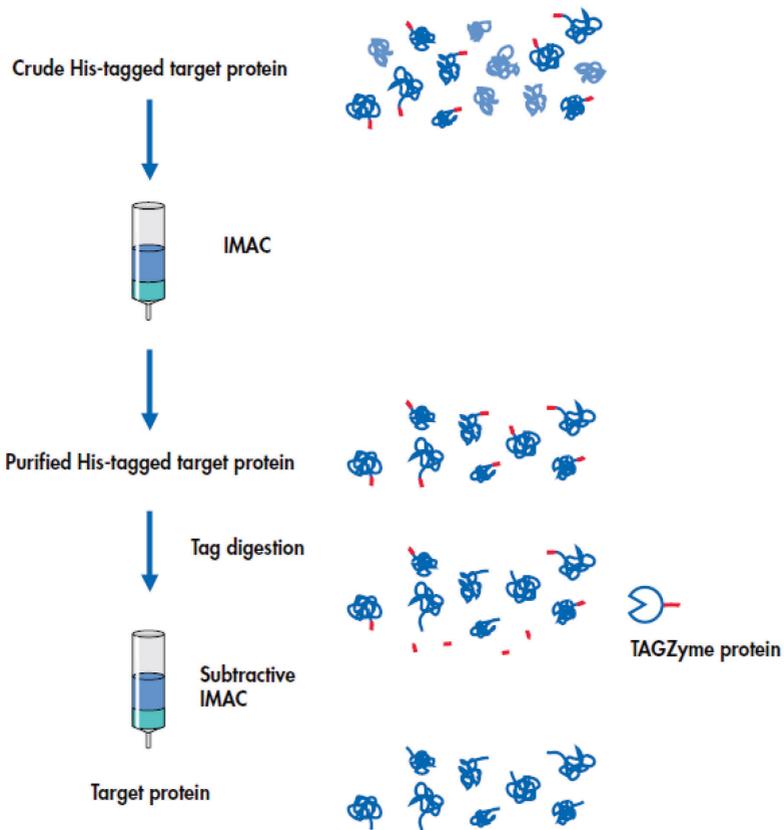


Figure 1. Schematic summary of the TAGZyme procedure.

The TAGZyme Principle

The TAGZyme System makes use of a highly specific and processive exoproteolytic cleavage of N-terminal amino acids for the removal of small affinity tags from proteins (e.g., 6xHis tags). After completion of the digestion, the exoproteolytic enzyme—which carries His tags at the C-terminus—is removed by subtractive IMAC. The efficiency of the total procedure is supplemented by the use of a series of *E. coli* expression vector, TAGZyme pQE-2 Vector DNA. This expression vector combines the high-level expression of 6xHis-tagged proteins delivered by the pQE vector series with specially designed His tag-coding and multiple cloning site sequences that allow complete and convenient removal of N-terminal His tags. The vector has been optimized for use with the TAGZyme System. Occasionally, problems may be encountered when processing other tags. For an overview of the suitability of other tags for TAGZyme processing; please consult Table 2 (page 17). N-terminal amino acids are cleaved off as dipeptides by dipeptidyl aminopeptidase I (DAPase Enzyme, Figure 2). After removal of the first N-terminal dipeptide, the enzyme progressively cleaves off dipeptides until a stop point is encountered. Stop points are certain amino acids in defined positions within a dipeptide (see Table 1). As the His tag of TAGZyme enzyme appears at the C-terminus, they are not subject to digestion.

Table 1. DAPase stop points

Amino acid	DAPase stop point (↓) sequence*
Lysine (Lys, K)	Xaa-Xaa...Xaa-Xaa ↓ <u>Lys</u> -Xaa Xaa-Xaa...
Arginine (Arg, R)	Xaa-Xaa...Xaa-Xaa ↓ <u>Arg</u> -Xaa Xaa-Xaa...
Proline (Pro, P)	Xaa-Xaa...Xaa-Xaa ↓ Xaa-Xaa <u>Pro</u> -Xaa...
Proline (Pro, P)	Xaa-Xaa...Xaa-Xaa ↓ Xaa- <u>Pro</u> Xaa-Xaa...
Glutamine (Gln, Q)	Xaa-Xaa...Xaa-Xaa ↓ <u>Gln</u> [†] -Xaa Xaa-Xaa...

* Natural DAPase stop points (↓) are the following amino acids in the given position within a dipeptide (dipeptides that are cleaved off are underlined).

† In the presence of excess Qcyclyase™ Enzyme.

Proteins with intrinsic DAPase stop points

A range of mature proteins have natural DAPase Enzyme stop points (Table 1) causing the enzyme to release its substrate, that is, the detagged mature protein, as soon as this amino acid occurs in the N-terminal position. Accordingly, N-terminal His tags containing an even number of amino acid residues can be completely and specifically removed by treatment with DAPase Enzyme alone (Figure 2). After digestion is complete, the C-terminally His-tagged DAPase Enzyme, residual undigested target protein, and copurified contaminants are removed by subtractive IMAC. The entire process is typically completed within 45 minutes.

Proteins with Intrinsic DAPase Stop Points

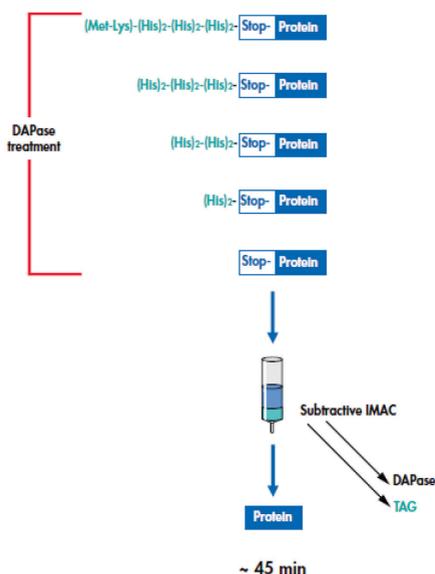


Figure 2. Combined cleavage and purification strategy. Procedure for proteins having an intrinsic DAPase stop point.

Applications for the TAGZyme System

The TAGZyme System is suitable for cleavage of every N-terminal His tag that contains certain sequence characteristics that are described in this handbook.

Therapeutic proteins

In the production of therapeutic proteins for use in humans or animals, it is often desirable to remove any artificial additions such as His tags.

The specificity and the speed of action of the recombinant enzymes in the TAGZyme System make it an ideal tool for production of recombinant proteinaceous therapeutics, where precisely defined cleavage characteristics are required. Due to the independence of this system from sequence-determined cleavage sites, the TAGZyme System provides the highest level of specificity of protein cleavage and target protein integrity. The TAGZyme System components are available in various formats including bulk quantities of single enzymes.

Moreover, the TAGZyme System can be combined with Ni-NTA-based IMAC protein purification strategies for which Drug Master Files (Type II) are available for support in regulatory affairs. The subtractive Ni-NTA IMAC processing steps within the TAGZyme procedure can contribute to an increase in the purity of the target protein.

For production where the highest safety standards are required, GMP-quality TAGZyme enzyme is available. Please contact QIAGEN for details.

Protein crystallography and structure determination

Structure determination of protein crystals has become more and more important in the context of drug screening and rational design of small molecules for protein ligands. To elucidate a protein's structure, it is often desirable to crystallize the protein in the native state, that is, without affinity tag additions.

Screening for crystallization conditions is often done in a high-throughput format. QIAGEN offers an automated solution for the production of large amounts of His-tagged protein for this purpose in 96-well format. TAGZyme is an ideal supplementary tool for this automated protein purification. Please contact QIAGEN for further details.

Protein microarrays

Depending on the chemistry of coupling of a purified protein to the surface of a chip and on the chip's intended application, it may be desirable to remove His tags from proteins prior to immobilization.

In combination with an automated solution for the purification of His-tagged proteins in a high-throughput format, TAGZyme is the method of choice to produce a large number of authentic, detagged proteins in a convenient and timesaving procedure.

Please contact QIAGEN for details of available solutions in automated high-throughput protein purification and downstream processing.

Basic research

Because the TAGZyme System is available in a small kit size and protocols are provided for the processing of a large range of proteins, the system is equally suited to His-tag removal from proteins used in small assays in basic research.

TAGZyme pQE Vectors

The TAGZyme pQE-2 Vector DNA is based on the pQE-80 vectors and is used for expression of N-terminally 6xHis-tagged proteins in *E. coli*. The His tag has been modified to encode the epitope Met-Lys-His₆ (MKH₆) a short His tag and the N-terminus Met-Lys motif that delivers a high expression level and lowest level of methionine processing in *E. coli* (1). Furthermore, in the rare case of methionine processing in *E. coli*, the N-terminal lysine residue would act as a DAPase stop point (see Table 1, page 8) and prevent DAPase from out-of-frame digestion. The presence of this DAPase stop point leaves the protein intact allowing removal of the protein by subtractive IMAC.

QIAexpress pQE vectors

High-level expression of 6xHis-tagged proteins in *E. coli* using the QIAexpress pQE vectors is based on the T5 promoter transcription-translation system. pQE plasmids belong to the pDS family of plasmids (2) and were derived from plasmids pDS56/RBSII and pDS781/RBSII-DHFRS (3). These low-copy plasmids (Figure 3) have the following features:

- Optimized promoter-operator element consisting of phage T5 promoter (recognized by the *E. coli* RNA polymerase) and two lac operator sequences which increase lac repressor binding and ensure efficient repression of the powerful T5 promoter
- Synthetic ribosomal binding site, RBSII, for high translation rates
- 6xHis-tag coding sequence either 5' or 3' to the cloning region
- Multiple cloning site and translational stop codons in all reading frames for convenient preparation of expression constructs
- Two strong transcriptional terminators: *t* from phage lambda (4), and T1 from the *rnmB* operon of *E. coli*, to prevent read-through transcription and ensure stability of the expression construct
- β -Lactamase gene (*bla*) confers resistance to ampicillin (5) at 100 μ g/ml (the chloramphenicol acetyl transferase gene (CAT) present between *t* and T1 has no promoter and is not normally expressed)

- ColE1 origin of replication (5)

Restriction map and sequence for the cloning region of the pQE-2 vector is presented in Figure 4. **Error! Reference source not found.** The entire sequence information is available at www.qiagen.com/TAGzyme-System. A strategy for the use of the pQE-TriSystem Vector for protein expression in insect and mammalian cells in combination with the TAGZyme System is outlined in the Appendix B, page 36.

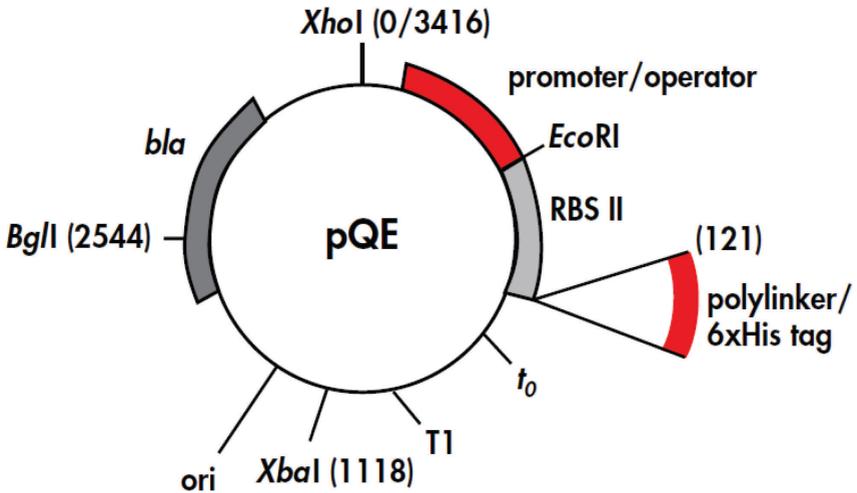


Figure 3. pQE vectors.

Regulation of expression — pREP4 plasmid

The extremely high transcription rate initiated at the T5 promoter can only be efficiently regulated and repressed by the presence of high levels of the lac repressor protein. *E. coli* host strains used in the QIAexpress System use a lac repressor gene in trans or cis to the gene to be expressed. In the trans system, the host strains contain the low-copy plasmid pREP4 which confers kanamycin resistance and constitutively expresses the lac repressor protein encoded by the lac I gene (6). The pREP4 plasmid is derived from pACYC and contains the p15A replicon. Multiple copies of pREP4 are present in the host cells that ensure the production of high levels of the lac repressor protein that binds to the operator sequences and tightly regulates recombinant protein expression. The pREP4 plasmid is compatible with all plasmids carrying the ColE1 origin of replication and is maintained in *E. coli* in the presence of kanamycin at a concentration of 25 µg/ml. The cis-repressed vectors pQE-80L, -81L, and -82L contain the lacIq gene and do not require the presence of pREP4.

Expression of recombinant proteins encoded by pQE vectors is rapidly induced by the addition of isopropyl-β-D-thiogalactoside (IPTG) which binds to the lac repressor protein and inactivates it. Once the lac repressor is inactivated, the host cell's RNA polymerase can transcribe the sequences downstream from the promoter. The transcripts produced are then translated into the recombinant protein. The special "double operator" system in the pQE expression vectors, in combination with the high levels of the lac repressor protein generated by pREP4 or the *lacI^q* gene on pQE-80L, pQE-81L, or pQE-82L, ensure tight control at the transcriptional level. The pREP4 plasmid is already present in the QIAexpress *E. coli* strains M15[pREP4] and SG13009[pREP4]. Using pQE-80L, pQE-81L, or pQE-82L with the cis-*lacI^q* gene, expression rates are comparable with those obtained using pQE-30, pQE-31, or pQE-32 vectors in combination with pREP4.

E. coli host strains

Any *E. coli* host strain containing both the expression (pQE) and the repressor (pREP4) plasmids can be used for the production of recombinant proteins. The QIAexpress System uses *E. coli* strain M15[pREP4] which permits high-level expression and is easy to handle. Strain SG13009[pREP4] (7) is also supplied and may be useful for the production of proteins that are poorly expressed in M15[pREP4]. Both the M15 and SG13009 strains are derived from *E. coli* K12 and have the phenotype Nal^s , Str^s , Rif^s , Thi^- , Lac^- , Ara^+ , Gal^+ , Mtl^- , F^- , RecA^+ , Uvr^+ , and Lon^+ .

E. coli strains that harbor the *lac^H* mutation, such as XL1 Blue, JM109, and TG1, produce enough lac repressor to efficiently block transcription and are ideal for storing and propagating pQE plasmids. These strains can also be used as expression hosts for expressing nontoxic proteins, but they may be less efficient than the M15[pREP4] strain, and expression is regulated less tightly than in strains harboring the pREP4 plasmid. If the expressed protein is toxic to the cell, "leaky" expression before induction may result in poor culture growth or in the selection of deletion mutants that grow faster than bacteria containing the correct plasmid. Note that *E. coli* strains M15 and SG13009 do not harbor a chromosomal copy of the *lac^H* mutation, so pREP4 must be maintained by selection for kanamycin resistance. The cis-repressed pQE-80L series of vectors can be easily used with any *E. coli* host strain and kanamycin selection is not necessary.

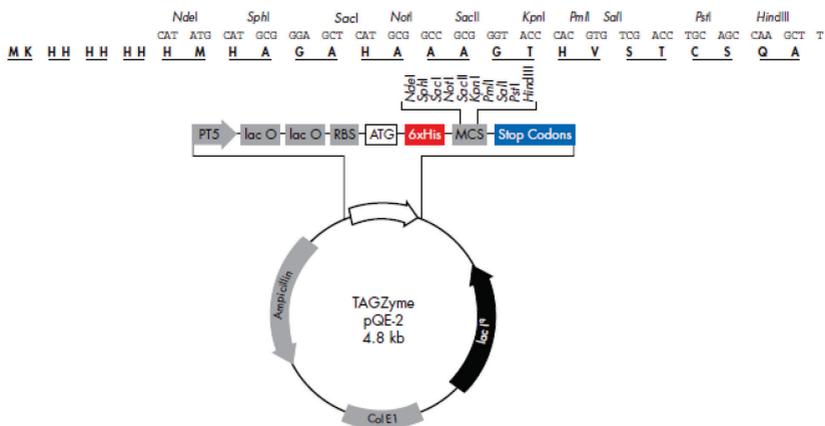


Figure 4. TAGZyme pQE vectors for N-terminal His tag constructs which facilitate exoproteolytic removal of N-terminal residues by TAGZyme enzymes. Restriction sites within the multiple cloning sites, DNA sequences, and the corresponding N-terminal amino acid sequences are shown. DAPase Enzyme cleaves off the underlined dipeptides until an introduced stop point is reached.

Refer to *The QIAexpressionist* (8) or to www.qiagen.com for further details and sequences of the TAGZyme pQE-2 Vector DNA.

The Cloning Procedure

The cleavage activity of DAPase Enzyme is dependent upon the amino acid composition of the His tag to be digested, that is, of the dipeptides to be cleaved. The TAGZyme pQE *E. coli* expression vectors have been specially designed to provide reliable and efficient His tag cleavage.

The TAGZyme pQE-2 tag sequences allow removal of the complete N-terminal tag irrespective of the cloning site of the DNA insert.

Table 2. Examples of the cleavage rates of various dipeptides by DAPase Enzyme

Rapid rate of cleavage	Medium rate of cleavage	Slow rate of cleavage	No cleavage = DAPase stop point
Xaa – Arg	Xaa – Asp*	Xaa – Phe [†]	Arg – Xaa
Xaa – Lys	Xaa – Glu*	Gly – Met	Lys – Xaa
His – Ala	Phe – Xaa [†]	Gly – Ser	Xaa – Pro
His – Gln	Ala – Ala	Ser – Met	Xaa – Xbb – Pro
His – Gly	Asp – Asp* [‡]		Gln – Xaa [§]
His – His	Glu – Glu* [‡]		
His – Met	Glu – His*		
Ala – His	Gly – Phe [†]		
Gly – His	Ser – Tyr		
Met – His	Ser – Thr		
His – Val	Gly – Ala		
	Gly – Thr		

* Positively or negatively charged side chains inhibit DAPase Enzyme cleavage, and sequences containing aspartic acid (Asp) or glutamic acid (Glu) can only be digested by DAPase Enzyme at acidic pH while sequences containing histidine (His) residues require a pH above 6.0. Therefore, 6xHis tags containing Glu or Asp can only be digested by DAPase Enzyme at a pH between 6.0 and 6.5.

[†] With a few exceptions, dipeptides containing Phe, Ile, Leu, Tyr, and Trp in either of the two positions of the dipeptide, are subject to slow cleavage.

[‡] Medium to slow cleavage rate.

[§] Gln-Xaa serves as a stop point for DAPase Enzyme only in the presence of Qcyclase Enzyme, which converts the glutamine residue to pyroglutamate (which can be removed by the action of pGAPase™ Enzyme).

The use of expression vectors other than TAGZyme pQE constructs in conjunction with TAGZyme enzymes is possible. However, since these vectors have not been specifically designed for use with the TAGZyme System, the amino acid sequence to be cleaved off should be carefully analyzed for efficiency of dipeptide cleavage using Table 2. It is also recommended to make use of Table 2 and the guidelines given below whenever a new N-terminal tag is designed for cleavage by DAPase Enzyme. The TAGZyme System is equally suited for use with prokaryotic and eukaryotic expression systems.

In addition to considering the dipeptide composition within the N-terminus to be cleaved, the following guidelines should be followed to achieve successful removal of His tags from recombinant proteins using the TAGZyme System.

- The total number of amino acid residues in the tag to be cleaved off should not exceed 30.
- If the target protein contains an intrinsic DAPase stop point (see Table 1) which is to be utilized, the tag must be composed of an even number of residues.
- Arginine (R) and lysine (K) residues must not be placed in odd-numbered positions of the tag.
- The tag sequence must not contain any proline (P) residues.
- The N-terminal sequence of the tag should be designed to minimize in vivo processing of the N-terminal methionine residue.

Processing of methionine leads to out-of-frame DAPase digestion and results in the accumulation of erroneously processed products. In *E. coli*, the residue adjacent to the initial methionine influences the degree of processing, and a bulky residue should be placed in this position. We recommend lysine (found in TAGZyme pQE-2 Vector DNA), but arginine, glutamate, and aspartate are also effective as a penultimate residue that reduces methionine processing in *E. coli* (1). Please note that DAPase Enzyme treatment must be carried out at pH 6.0–6.5 if aspartate or glutamate residues occur in the His-tag sequence to be cleaved (see Table 2 and Table 3).

Table 3. Characteristics of recommended His tag N-termini

N-terminus	Level of Methionine processing in <i>E. coli</i> *	Expression level in <i>E. coli</i> *	DAPase Enzyme cleavage rate	Other advantages
Met ¹ Lys ² – His tag	Very low	Very high	Rapid	DAPase Enzyme stop point for out-of-frame digestion†
Met ¹ Arg ² – His tag	Low	Very high	Rapid	DAPase Enzyme stop point for out-of-frame digestion†
Met ¹ Glu ² – His tag	Low	High	Medium (only at acidic pH)	
Met ¹ Asp ² – His tag	Low	High	Medium (only at acidic pH)	

* See reference 1 and 11.

† See reference 1.

‡ See also Table 1.

In general, the nucleotide sequence encoding the tag has a strong influence on the expression level and self-complimentary mRNA sequences close to the ATG start codon should be avoided. Furthermore, it has been observed that the amino acids lysine (codon AAA) and arginine (e.g., codon CGT) significantly improve expression if placed next to the start methionine coded by ATG.

The tag sequence should be designed to avoid dipeptides that result in slow DAPase cleavage rates. The rate at which DAPase Enzyme cleaves off different dipeptides from the N-terminus depends on the amino acid residues in the dipeptide. Dipeptides containing an arginine or lysine residue in the C-terminal position are cleaved off very quickly, whereas dipeptides containing a hydrophobic amino acid residue (i.e., Phe, Ile, Leu, Tyr, Trp) are cleaved off relatively slowly. Examples of cleavage rates with respect to dipeptide composition are shown in Table 2, page 17.

Proteins expressed using other pQE vectors that encode N-terminal His tags are poorly processed as they encode the sequence Met-Arg-Gly-Ser. The second dipeptide, Gly-Ser, is slowly cleaved by DAPase Enzyme (see Table 2). Furthermore, C-terminal His tags encoded by the pQE vectors pQE-60, -70, -16, and -TriSystem are not removed by the TAGZyme System, as the exoprotease cleaves dipeptides from the N-terminus of proteins.

In some cases, in vivo His-tag cleavage can occur, leading to erroneously processed products. Such processing may be observed when arginine residues appear in the middle of His-tag sequence or close to the target protein sequence. It is recommended that arginine residues only appear close to the N-terminus of the tag.

In response to out-of-frame digestion resulting from methionine processing or in vivo cleavage of the tag, incorporation of DAPase stop points in the tag is recommended (see above).

Erroneously processed products with two or more consecutive histidine residues remaining can be efficiently removed by subtractive Ni-NTA IMAC after DAPase digestion. It is recommended to place arginine in the N-terminal sequence of a tag (see above).

Cleavage Protocols

Notes before starting

His-tag design

Please note that the precise amino acid sequence of a polyhistidine tag and the nucleotide sequence selected to encode it are of great importance for the overall performance of the resulting construct during expression, post-translational processing, purification, and tag removal. For these reasons, we strongly recommend using the pQE-TAGZyme expression vector that has been optimized for use with the TAGZyme System. If other vectors are used, please follow the guidelines given in this handbook regarding tag design and gene construction strategy.

Expression, Ni-NTA purification, and desalting of His-tagged proteins

For expression and purification of 6xHis-tagged proteins refer to *The QIAexpressionist*, a handbook for high-level expression and purification of 6xHis-tagged proteins (8).

The TAGZyme procedure works well at pH 6.3–7.5 in the absence of imidazole. Because an addition of imidazole or a reduction in pH are usually used for elution of His-tagged proteins from Ni-NTA matrices, the purified target should be desalted before starting the TAGZyme procedure. Desalting can be performed using standard techniques such as gel filtration, ultrafiltration, hollow-fiber modules, or dialysis.

The presence of imidazole leads to a decrease in the cleavage rate, probably due to competitive inhibitory effect on DAPase catalysis. Simple dilution of IMAC elution fractions for direct use in the TAGZyme procedure without prior desalting may be possible but reaction parameters must be optimized in pilot experiments. Imidazole should be diluted to a concentration of 50 mM or lower. The final protein concentration should not be lower than 0.3 mg/ml.

Buffers and enzyme activity

Phosphate buffers are recommended for use with the TAGZyme System due to their low tendency to strip metal ions from IMAC matrices. However, other buffers may be used such as Tris, Bis-Tris, Bis-Tris-Propane, and MES. For further details on the compatibility of reagents with Ni-NTA IMAC matrices and procedures, refer to Table 4 in *The QIAexpressionist* (8) or visit www.qiagen.com.

The optimal pH for TAGZyme catalyzed removal of tags, and also for removal of His-tagged contaminants using subtractive IMAC, is 6.7 to 7.5. The DAPase Enzyme works well between 4 and 37°C as outlined in Table 4.

Table 4. Effect of reaction temperature on DAPase activity

Reaction temperature	Relative activity of DAPase Enzyme
37°C	100%
Room temperature (20–25°C)	50%
4–6°C	10%

DAPase Enzyme

DAPase Enzyme is inactive in the presence of denaturants (guanidine hydrochloride or urea), even at low concentrations. DAPase Enzyme is 85–100% active in the presence of up to 0.4% CHAPS, Tween® 20, Tween 80, and Triton® X-100 and 60–70% active in the presence of 1% of these detergents.

The enzyme has a broad pH optimum between pH 4 and 8. However, the activity depends on the dipeptides to be cleaved off (see Table 2 and Table 3). The optimal pH of DAPase-catalyzed removal of His tags is pH 6.7 to 7.2. The enzyme's activity is approximately 50% of maximum at pH 6.3 and 7.5 and approximately 10% at pH 5.8 and 8.0. DAPase Enzyme must be activated before use by the reducing agent cysteamine (see protocols).

TAGZyme Buffer provides optimal TAGZyme enzyme activity conditions and its use is recommended in TAGZyme procedures.

Cysteamine activation

Cysteamine is used as an activator for DAPase Enzyme. It is thought that at a physiological pH, cysteamine, and its oxidized form cysteamine, act as a hydrogen donor for the reduction of disulfides in the enzymes.

Stepwise Use of the TAGZyme System

Normally, by following the recommendations below, sufficient cleavage of the target protein will be obtained. However, the actual rate of cleavage of an N-terminal His tag may vary from protein to protein. This can be due to variations in steric accessibility of the tag or due to modifications of amino acid residues. If the optimal cleavage conditions have to be determined for a specific purification process, it is recommended that a pilot DAPase digestion reaction be carried out.

Therefore, the use of the TAGZyme System should include the following steps:

- Pilot digestion to establish optimum enzyme concentrations
- Preparative DAPase digestion
- Subtractive IMAC using Ni-NTA resin

Analysis during the TAGZyme procedure

We recommend that the efficiency of cleavage reactions is checked by subjecting samples from the different steps of the TAGZyme procedure to SDS-PAGE analysis.

Cleavage of N-terminal 6xHis tags using DAPase Enzyme

The data in Table 5 can be used as a rough guide to the amount of DAPase Enzyme necessary to completely digest the N-terminal tag of a recombinant protein. However, substantial differences in activity may be encountered from protein to protein (see above). Therefore, the figures in Table 5 should be considered as a starting point for the pilot experiments only, and not as a general rule for any protein and reaction scale.

Table 5. Times generally required for DAPase digestion at the given DAPase Enzyme-to-target protein ratio

Amount of target protein	Amount of DAPase Enzyme	Reaction temperature	Required reaction time
1 mg	50 mU	4°C	5 h
50 µg	2.5 mU	20–25°C	60 min
		37°C	60 min

The following section is for DAPase digestion of tagged proteins containing an intrinsic stop point (see above).

Buffer preparation

From a 10x stock solution (see Appendix) prepare enough 1x TAGZyme Buffer (20 mM sodium phosphate, pH 7.0; 150 mM NaCl) for desalting and the TAGZyme digestion. Approximately 50 ml 1x TAGZyme Buffer is sufficient for a small-scale experiment.

Note that tags containing glutamate or aspartate residues can only be efficiently cleaved at pH 6.0–6.5. In this case, prepare the TAGZyme Buffer at lower pH (20 mM sodium phosphate, pH 6.0–6.5; 150 mM NaCl).

Desalting

Desalt your His-tagged protein using gel filtration, ultrafiltration dialysis, hollow-fiber filtration, or the method best suited to your needs. It is recommended that proteins are exchanged into 1x TAGZyme Buffer.

Ni-NTA matrices show negligible leaching of nickel ions into the elution buffer (10). However, when using IMAC resins that bind metals weaklier, addition of 5–10 mM EDTA to the eluted protein fraction prior to the desalting procedure is recommended to remove nickel ions from the His tag.

Protocol 1: DAPase Digestion (Small-Scale Pilot Experiment) for Proteins Containing Intrinsic DAPase Stop Points

The amount of DAPase Enzyme and the length of incubation time required for complete digestion of the tag depend on the target protein and its concentration. Generally, digestion is complete after treatment for 30 minutes at 37°C with 50 mU DAPase Enzyme per mg protein but the optimal conditions for the processing of a His-tagged protein must be determined in pilot experiments. Table 6 gives guidelines for the conditions required for digestion of 50 µg target protein in TAGZyme buffer. Protein concentration should be at least 0.3 mg/ml. The reaction should take place in a volume of 50 to 300 µl. Larger reaction volumes require longer incubation times due to the dilution of the reactants.

Table 6. Recommendations for DAPase Enzyme digestion pilot experiments

Enzyme/reagent	For processing of 50 µg His-tagged target protein	
	Starting point	Titration range
DAPase Enzyme (1 U/ml)*	2.5 mU (2.5 µl)	0.5–5 mU (0.5–5 µl)
Cysteamine·HCl (2 mM)*	5 µl	1–10 µl

* Dilute an aliquot of the DAPase Enzyme and cysteamine stock solutions according to steps 1 and 2 of protocol 1.

It is recommended that the progress of each digestion be analyzed by SDS-PAGE. Take 5 µl samples from each reaction every 10 minutes, add SDS sample buffer to inactivate the enzyme, and load samples onto an SDS gel including an undigested control. Alternatively, or in addition to varying the enzyme concentration, the incubation temperature can be varied.

Procedure

1. Prepare a 1/10 dilution of DAPase Enzyme stock solution (10 U/ml) using 1x TAGZyme Buffer.
2. Prepare a 1/10 dilution of cysteamine·HCl stock solution (20 mM) using distilled water.
3. Mix 1 volume DAPase Enzyme solution (1 U/ml) with 2 volumes cysteamine·HCl (2mM). DAPase Enzyme is activated by cysteamine in this step.
4. Incubate for 5 min at room temperature. The enzyme mixture must be used within 15 min of preparation.

During DAPase Enzyme preparation, bring the protein solution to the desired incubation temperature (4–37°C).

5. Start the digestion by adding the enzyme mix to the temperature-adjusted protein solution.
6. Incubate for a length of time that should deliver complete digestion (see Table 5). If desired, take samples during the reaction for SDS-PAGE analysis.

If removal of DAPase Enzyme is required, please refer to the protocol for small scale subtractive IMAC in the Appendix, page 39.

Protocol 2: Preparative DAPase Digestion for Proteins Containing Intrinsic DAPase Stop Points

The TAGZyme Kit has been designed to enable processing and purification of at least 10 mg of desalted and IMAC-purified tagged protein. Suitable protein concentrations in digestion reactions are between 0.3 and 5 mg/ml. The amount of DAPase Enzyme (mU) to be used for digestion per mg of desalted protein is established in the pilot DAPase digestion experiment. Table 7 lists the corresponding volumes to be used in a preparative digestion per mg of 6xHis-tagged protein.

Table 7. Recommendations for preparative DAPase digestion

Enzyme/reagent	For processing of 50 µg His-tagged target protein	
	Starting point	Titration range
DAPase Enzyme (10 U/ml)*	50 mU (5 µl)	10–100 mU (1–10 µl)
Cysteamine-HCl (20 mM)*	5 µl	1–10 µl

In most cases, a linear scaling up of the conditions found to give complete digestion in the pilot experiment will give satisfactory results in a preparative digestion. If desired, a time course can be carried out to verify the optimal digestion conditions determined in the pilot experiments.

Procedure

1. Mix 1 volume DAPase Enzyme solution (10 U/ml) with 1 volume cysteamine-HCl (20 mM).

DAPase Enzyme is activated by cysteamine in this step.

2. Incubate for 5 min at room temperature. The enzyme mixture must be used within 15 min of preparation.

During DAPase Enzyme preparation, bring the protein solution to the desired incubation temperature (4–37°C).

3. Start the digestion by adding the enzyme mix to the temperature-adjusted protein solution.
4. Incubate for a length of time that was found to be sufficient for complete digestion in the pilot experiment. If desired, take samples during the reaction for SDS-PAGE analysis.

Protocol 3: Removal of DAPase Enzyme by Subtractive IMAC

This protocol applies to a cleavage reaction containing 1 mg target protein. Refer to Table 11, page 39, for Ni-NTA IMAC scale up recommendations.

Procedure

1. Resuspend the Ni-NTA Agarose suspension by shaking and pipet 1 ml of the 50% suspension (corresponding to 0.5 ml bed volume) into one of the disposable columns provided in the kit.

Alternatively, pack an LC or FPLC® column with 0.5 ml bed volume of Ni-NTA Agarose.*

2. Equilibrate the column with 5 ml (10 column volumes) 1x TAGZyme Buffer.

Steps 1 and 2 can be performed during enzyme incubation. When using a chromatography system, do not exceed a flow rate of 0.5 ml/min to ensure quantitative binding of the His-tagged DAPase Enzyme.

3. Pass the digestion reaction mixture through the column and collect the flow-through fraction.

Important: The flow-through fraction contains the processed native protein.

4. Wash the column with 2 ml of 1x TAGZyme Buffer or until the flow-through no longer contains protein. Collect the fraction(s).

Important: The flow-through fractions contain the processed native protein.

5. Add the flow-through fraction from step 3 to the protein-containing fractions from step 4. Discard the Ni-NTA resin used in this subtractive step.

The TAGZyme procedure is finished and the detagged native protein is ready for further applications.

* An HR 5/2 or HR 5/5 FPLC column (Amersham Pharmacia Biotech) is suitable for such a volume of matrix. For processing of larger amounts of protein please refer to the bed-volume size and flow-rate recommendations on page 40. In general, column height should be $\geq 2x$ column diameter.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit support.qiagen.com).

Comments and suggestions

End product not sufficiently pure

- | | |
|--|--|
| a) Poor quality of tagged protein preparation | Refer to <i>The QIAexpressionist</i> for guidelines and limitations of Ni-NTA IMAC purification of His-tagged proteins. |
| b) Inefficient digestion | <p>When working with proteins purified by IMAC using matrices other than Ni-NTA, metal ions may be leached from the matrix. These metal ions may have an inhibitory effect on digestion. Include EDTA in the desalting procedure prior to digestion.</p> <p>Check N-terminal amino acid sequence for inefficiently cleaved dipeptides (Table 2, page 17). Check for the presence of DAPase Enzyme stop points in odd-numbered positions in the protein sequence. Check whether pH of the reaction is correct. The pH must be lowered when Glu or Asp residues are in the tag sequence to be cleaved.</p> <p>Check the pH of the 1x TAGzyme Buffer preparation. pH optimum for the TAGzyme procedure is pH 6.7. to 7.5.</p> <p>Check for the presence of denaturants. Even low concentrations will inactivate DAPase Enzyme.</p> <p>Be sure that the length of the tag to be cleaved does not exceed 30 amino acids.</p> <p>The optimal DAPase Enzyme-to-protein ratio may vary from protein to protein. Determine optimum conditions as described in pilot experiments. In some cases, a certain percentage of the tagged protein is not cleaved, e.g., due to steric hindrance. This residual His-tagged protein will be efficiently removed by subtractive IMAC.</p> |
| c) Too many amino acids removed (DAPase digestion into the body of the target protein) | DAPase stop point is not present at correct position. Check sequence for correct positioning of stop continues point (Table 1, page 8). |

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Appendix A: Enzyme Specifications

DAPase Enzyme

Recombinant dipeptidyl peptidase I, polyhistidine-tagged (DPPI/dipeptidyl aminopeptidase I/DAPI/Cathepsin C; EC 3.4.14.1).

Source	Recombinant baculovirus expression vector system expressing the cloned DPPI gene from rat liver
Supplied form	Solution in 3 mM sodium phosphate; 150 mM NaCl; 2 mM cysteamine-HCl; 50% glycerol, pH 6.7–7.0
Purity	>98% (determined by SDS-PAGE)
Concentration	10 units/ml
Storage	–20°C
Stability	9 months at –20°C; 1 week at 2–8°C
Assay conditions	DAPase Enzyme is assayed at 37°C in 20 mM citric acid, 150 mM NaCl, 1 mM EDTA, 5 mM cysteamine, pH 4.5, containing 4 mM Gly-Phe-p-nitroanilide as substrate.
Unit definition	One unit is defined as the amount of enzyme that converts 1 μ mol of substrate per minute under the conditions used.
Specific activity	7–11 unit/mg protein (protein determined by Bradford assay using BSA as standard)

Solutions required

10x TAGZyme Buffer	For 500 ml
200 mM NaH ₂ PO ₄	13.80 g NaH ₂ PO ₄ ·H ₂ O (MW 137.99 g/mol)
1.5 M NaCl	43.83 g NaCl (MW 58.44 g/mol)

Adjust to desired pH with NaOH. Filter (0.2 μ m) into a sterile storage vessel and store at 2–8°C.

Note: Upon 1:10 dilution pH will shift from 6.3 to 7.0.

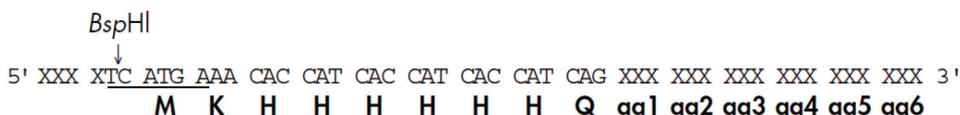
Appendix B: Recommended PCR Cloning Strategy for Use of the Vector pQE-TriSystem in Combination with TAGZyme Cleavage

The pQE-TriSystem Vector allows parallel expression of a His-tagged recombinant protein in *E. coli*, baculovirus-infected insect cells, and mammalian cells using a single construct. The vector encodes a C-terminal His tag (which cannot be removed by the TAGZyme System). To make use of this extremely versatile expression vector, an N-terminal His tag must be introduced by PCR. A cloning strategy and primer design recommendations are given below.

- Clone into the most N-terminal restriction site, *Nco*I, to avoid the introduction of slowly cleaved dipeptides (see Table 2, page 17).
- Design a 5' (sense) oligonucleotide primer including the *Nco*I-compatible 5' restriction site BspHI and including sequences coding for a 6xHis tag (the use of the BspHI site allows the insertion of a lysine residue as the next amino acid following the start methionine).
- Include a C-terminal stop codon (UAA) after the last amino acid
- Clone into any one of the C-terminal restriction sites (e. g., HindIII)

Refer to *The Q/Aexpressionist* (8) or visit us at www.qiagen.com for more details on the pQE-TriSystem vector.

Design of the 5' (sense) PCR primer with an introduced glutamine stop point*



* If the protein to be expressed contains a natural stop point within the amino acids aa1 – aa3 (see Table 1, page 9) this can be used as a DAPase Enzyme stop point instead of the glutamine residue.

Design of the 3' (antisense) PCR primer including a stop codon (TTA) for cloning into the *Hind*III site

Cloning into restriction sites located 3' of *Nco*I is not recommended, because this will result in the insertion of an alanine residue directly behind the initial methionine. Alanine does not efficiently prevent methionine processing (1) which may result in erroneously processed DAPase Enzyme digestion products. Furthermore, the second N-terminal dipeptide would be Ile-Ser which will be inefficiently cleaved (see Table 2, page 17).

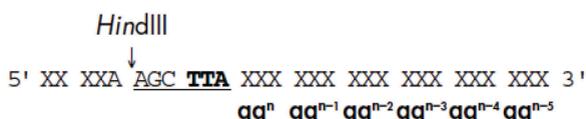


Table 8. The genetic code

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Table 9. *E. coli* codon usage table

First position (5' end)	Second position								Third position (3' end)
	U		C		A		G		
U	2.23	Phe	0.85	Ser	1.62	Tyr	0.52	Cys	U
	1.66	Phe	0.86	Ser	1.22	Tyr	0.65	Cys	C
	1.39	Leu	0.72	Ser	0.20	Stop	0.09	Stop	A
	1.36	Leu	0.89	Ser	0.02	Stop	1.52	Trp	G
C	1.10	Leu	0.70	Pro	1.29	His	2.09	Arg	U
	1.11	Leu	0.55	Pro	0.97	His	2.20	Arg	C
	0.39	Leu	0.84	Pro	1.53	Gln	0.36	Arg	A
	5.26	Leu	2.32	Pro	2.88	Gln	0.54	Arg	G
A	3.03	Ile	0.90	Thr	1.77	Asn	0.88	Ser	U
	2.51	Ile	2.34	Thr	2.17	Asn	1.61	Ser	C
	0.44	Ile	0.71	Thr	3.36	Lys	0.21	Arg	A
	2.53	Met	1.44	Thr	1.03	Lys	0.12	Arg	G
G	1.83	Val	1.53	Ala	3.21	Asp	2.47	Gly	U
	1.53	Val	2.55	Ala	1.91	Asp	2.96	Gly	C
	1.09	Val	2.01	Ala	3.94	Glu	0.80	Gly	A
	2.59	Val	3.36	Ala	1.78	Glu	1.11	Gly	G

Note: For each codon, frequency of usage (%) and the corresponding amino acid are given.

His tags suitable for exoproteolytic cleavage by the TAGZyme System

The following His tags have been shown to deliver excellent expression rates in *E. coli* and be well-suited for convenient cleavage using the TAGZyme System. For a more detailed overview, see reference 12.

Table 10. Amino acid and encoding nucleotide sequences for recommended His tags

	1	2	3	4	5	6	7	8	9
Amino acid	M	K	H	H	H	H	H	H	(Q)
Codon	ATG	AAA	CAT	CAC	CAT	CAC	CAT	CAC	(QCT)
Amino acid	M	R	H	H	H	H	H	H	(Q)
Codon	ATG	CGT	CAT	CAC	CAT	CAC	CAT	CAC	(QCT)

Small-scale subtractive IMAC

- Pipet 50 µl Ni-NTA suspension (25 µl settled bed volume) into a microcentrifuge tube and equilibrate by spinning briefly (10 s), removing the supernatant, adding 500 µl TAGZyme buffer, spinning briefly (10 s), and removing the supernatant.
- Add the TAGZyme Enzyme reaction mixture and incubate at 4°C or room temperature for 10 min on a shaker platform or an end-over-end shaker. Ensure that the suspension is mixed well.
- To recover the target protein, spin briefly (10 s), and carefully remove the supernatant. Be sure not to disturb the Ni-NTA matrix, which contains the bound TAGZyme Enzyme.

Recommendations for subtractive IMAC scale-up

The use of a chromatography system with which the flow rate can be precisely controlled is recommended. When processing larger columns the flow rate is a critical factor for the efficient binding of His-tagged proteins to the Ni-NTA matrix. In addition, the use of Ni-NTA Superflow is recommended as it can withstand higher back pressures (up to 10 bar = 1 MPa). Table 11 gives guidelines for flow rates and column dimensions to be used when scaling up subtractive IMAC.

Table 11. Recommended column dimensions and flow rates for Ni-NTA IMAC scale up

Amount of protein to be processed	Ni-NTA bed volume	Column dimensions (internal diameter x length)	Flow rate (ml/min)		Linear flow rate (cm/h)	
			Load	Wash	Load	Wash
Up to 1 mg	0.5 ml	0.5 cm x 2.5 cm	0.5	1.0	150	300
50 mg	10 ml	1.6 cm x 6 cm	5	10	150	300
1 g	200 ml	5 cm x 10.2 cm	50	100	150	300

Ordering Information

Product	Contents	Cat. no.
TAGZyme Kit	0.5 Units DAPase Enzyme, 30 Units Qcyclase Enzyme, 10 Units pGAPase Enzyme, 20 mM Cysteamine-HCl (1 ml), Ni-NTA Agarose (10 ml), 20 Disposable Columns	34300
TAGZyme pQE Vector	TAGZyme pQE-2 Vector DNA, 25 µg	32932
TAGZyme DAPase Enzyme (2.5 U)	2.5 Units DAPase Enzyme, 20 mM Cysteamine-HCl (1 ml)	34362
TAGZyme DAPase Enzyme (50 U)*	50 Units DAPase Enzyme, 20 mM Cysteamine-HCl (25 ml)	34366
Related Products		
Ni-NTA Agarose [†] (25 ml)	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Ni-NTA Superflow [†] (25 ml)	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
Polypropylene Columns (1 ml)	50/pack, 1 ml capacity	34924

* Bulk enzyme quantities are customized products; delivery may take up to 6 weeks. Enzymes are also available in GMP-grade. Please inquire.

[†] Available in bulk quantities; please inquire.

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Document Revision History

Date	Changes
07/2015	Initial revision
12/2021	Updated the name of "Vector TAGZyme pQE-1" to "TAGZyme pQE-2 Vector DNA". Changed the name of Updated the following sections: Kit Contents, Storage, Introduction, The TAGZyme Principle, TAGZyme pQE Vectors, The Cloning Procedure, Cleavage Protocols, Analysis during the TAGZyme procedure, Troubleshooting, Appendix A, and Ordering Information. Also updated Figure 2 and Table 4. Removed the following sections: Qcyclase Enzyme; pGAPase Enzyme; Cleavage of N-Terminal His Tags Using DAPase, Qcyclase, and pGAPase Enzymes; Buffer preparation; Desalting; and Protocols 4–9. Also deleted Figure 5 and Table 8.

Notes

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