The QIAexpress System

The QIAexpress System provides materials for expression, purification, detection, and assay of 6xHis-tagged proteins. The QIAexpressionist covers expression and purification products (pQE vectors, host strains, and Ni-NTA chromatographic matrices) which allow the fast and efficient production and purification of heterologously expressed 6xHis-tagged proteins.

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 (Bujard *et al.* 1987) and were derived from plasmids pDS56/RBSII and pDS781/RBSII-DHFRS (Stüber *et al.* 1990). 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*₀ from phage lambda (Schwarz *et al.* 1987), and T1 from the *rrnB* operon of *E. coli*, to prevent read-through transcription and ensure stability of the expression construct
- β-lactamase gene (*bla*) confers resistance to ampicillin (Sutcliffe 1979) at 100 µg/ml (the chloramphenicol acetyl transferase gene (CAT) present between *t*₀ and T1 is not expressed)
- ColE1 origin of replication (Sutcliffe 1979)

Restriction maps and sequences for the cloning regions of QIAexpress vectors are presented in the appendix on page 117. The entire sequence information is available at www.qiagen.com.

*Figure 3. pQE vectors.*
QIAexpress pQE-TriSystem Vector for expression in *E. coli*, mammalian and insect cells

High-level expression of His-tagged proteins from a single vector is possible in three different expression systems due to the presence of the T5 promoter/lac operator transcription–translation system for expression in *E. coli*; the p10 promoter for baculovirus-based expression in insect cells; and the CAG (CMV/actin/globin) promoter for expression in mammalian cells. The pQE-TriSystem vector has the following features:

- An optimized promoter-operator element consisting of the coliphage T5 promoter (recognized by the *E. coli* RNA polymerase) and the lac operator sequence which binds lac repressor and ensures efficient repression of the powerful T5 promoter in *E. coli*.
- Synthetic bacterial ribosomal binding site for high translation rates.
- Strong, constitutive CAG promoter that mediates transient mammalian expression. It consists of the chicken β-actin promoter with the CMV immediate-early enhancer upstream, and a splicing acceptor site of the β-globin gene downstream.
- Downstream rabbit-globin–terminator sequence containing the signals for polyadenylation of the mRNA transcript.
- Kozak consensus sequence facilitating efficient translation initiation in mammalian systems.
- Late-very late p10 promoter for controlled recombinant protein expression in insect cells.
- Two segments derived from the open reading frames 603 and 1629 of *Autographa californica* nuclear polyhedrosis virus (AcNPV) genomic DNA that allow generation of recombinant baculovirus by homologous recombination.
- 8xHis-tag coding sequence 3′ of the cloning region (C-terminal His-tag)
- A multiple cloning site and translational stop codons in all reading frames for convenient preparation of expression constructs.
- Two strong transcription terminators — T7 from *E. coli* bacteriophage T7 and rabbit globin terminator region — to prevent read-through transcription and ensure stability of the expression construct.
- β-lactamase gene (*bla*) which confers resistance to ampicillin at 100 µg/ml in *E. coli*.
- pUC origin of replication, compatible with pREP4 in *E. coli*.

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 (see appendix, page 118) which confers kanamycin resistance and constitutively expresses the lac repressor protein encoded by the lacI gene (Farabaugh 1978). 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 which 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 lacI 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 lacIq 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-lacIq 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] (Gottesman et al. 1981) 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 derived from E. coli K12 and have the phenotype NaI+, Strr, Rifr, Thir, Lac−, Ara+, Gal+, Mfr−, F−, RecA+, Uvr+, Lon+.

E. coli strains that harbor the lacIq 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 which grow faster than bacteria containing the correct plasmid. Note that E. coli strains M15 and SG13009 do not harbor a chromosomal copy of the lacIq 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.
The 6xHis tag

The 6xHis affinity tag facilitates binding to Ni-NTA. Using pQE vectors it can be placed at the C- or N-terminus of the protein of interest. It is poorly immunogenic, and at pH 8.0 the tag is small, uncharged, and therefore does not generally affect secretion, compartmentalization, or folding of the fusion protein within the cell. In most cases, the 6xHis tag does not interfere with the structure or function of the purified protein as demonstrated for a wide variety of proteins, including enzymes, transcription factors, and vaccines. A further advantage of the 6xHis tag is that it allows the immobilization of the protein on metal-chelating surfaces such as Ni-NTA HisSorb Strips or Plates and therefore simplifies many types of protein interaction studies. In addition, Anti-His Antibodies can be used for detection.

Ni-NTA technology

Immobilized-metal affinity chromatography (IMAC) was first used to purify proteins in 1975 (Porath et al. 1975) using the chelating ligand iminodiacetic acid (IDA, Figure 4). IDA was charged with metal ions such as Zn$^{2+}$, Cu$^{2+}$, or Ni$^{2+}$, and then used to purify a variety of different proteins and peptides (Sulkowski 1985). IDA has only 3 metal-chelating sites and cannot tightly bind metal ions. Weak binding leads to ion leaching upon loading with strongly chelating proteins and peptides or during wash steps. This results in low yields, impure products, and metal-ion contamination of isolated proteins.

\[ \text{Figure 4. Comparison of the interactions of different metal chelate matrices with nickel ions.} \]
Nitrilotriacetic acid (NTA), exclusively available from QIAGEN, is a tetradequate chelating adsorbent developed at Hoffmann-La Roche that overcomes these problems. NTA (Figure 4) occupies four of the six ligand binding sites in the coordination sphere of the nickel ion, leaving two sites free to interact with the 6xHis tag (Figure 5). NTA binds metal ions far more stably than other available chelating resins (Hochuli 1989) and retains the ions under a wide variety of conditions, especially under stringent wash conditions. The unique, patented NTA matrices can therefore bind 6xHis-tagged proteins more tightly than IDA matrices, allowing the purification of proteins from less than 1% of the total protein preparation to more than 95% homogeneity in just one step (Janknecht et al. 1991).

Additional information on the interaction of 6xHis-tagged proteins with Ni-NTA matrices can be found in the section “Purification” beginning on page 63.

**Ni-NTA chromatographic materials**

QIAGEN supplies the following Ni-NTA matrices for the purification of 6xHis-tagged proteins:

**Ni-NTA Agarose**

Ni-NTA Agarose is composed of Ni-NTA coupled to Sepharose® CL-6B and offers high binding capacity and minimal nonspecific binding. This material has excellent handling properties for batch, column, and low-pressure FPLC®. The high surface concentration of the NTA ligand is sufficient for the binding of approximately 5–10 mg of 6xHis-tagged protein per milliliter of resin. Ni-NTA Agarose is very stable and easy to handle.