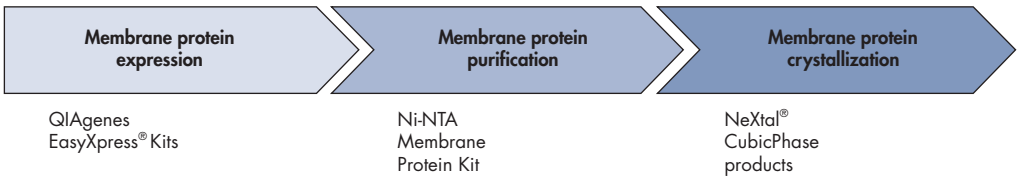
Complete solutions for membrane protein analysis

More than 30% of the proteins encoded in the human genome are membrane proteins. They are responsible for a variety of key biological functions such as communication between the cell and its environment, signal transduction, and nervous influx conduction. However, despite their important role, several aspects of the biology of membrane proteins are yet to be elucidated. Membrane proteins have various shapes and sizes, and they associate with the membrane in different ways. By nature they are hydrophobic and have a tendency to aggregate. They are often found as oligomers with a high molecular weight. These properties result in a range of technical difficulties when studying membrane proteins. QIAGEN has developed a portfolio of products dedicated for membrane protein analysis that provides optimized methods for expression, purification, and crystallization of membrane proteins.



Advanced technologies for membrane protein expression

Expression of membrane proteins is challenging and can involve tedious optimization to generate sufficient quantities of protein for further characterization. Often poorly expressed proteins can only be obtained in large amounts after repeated expression or costly scaled-up fermentations. Poor solubility can also be an issue, as recombinant proteins that are expressed at high levels in *E. coli* are often sequestered in insoluble aggregates known as inclusion bodies. Optimization can involve screening a large number of variants (e.g., individual domains, deletion mutants) using conventional in vivo methods — cloning sequences into expression plasmids, transforming, selecting, and growing bacteria — and is a time-consuming and labor-intensive procedure. QIAGEN's optimized, easy-to-use solutions for membrane protein expression enable greater time savings, allowing you to obtain results faster.

QIAgenes Expression Kits

For optimized expression of recombinant human proteins in insect and mammalian or *E. coli* systems as well as cell-free extracts

- Expression-optimized genes — powered by GENEART®
- Genomewide human ORFs ready cloned into expression vectors
- Synthetic cDNA with guaranteed sequence identity
- His tag for Ni-NTA affinity purification
- Easy ordering online through GeneGlobe® (www.qiagen.com/GeneGlobe)

QIAGEN®

Due to their optimized coding sequences and state-of-the-art expression vectors, QIAgenes Expression Kits significantly increase the expression success and yield compared to wild-type cDNAs, even for difficult targets such as membrane proteins (Figure 1). They are compatible with expression in *E.coli*, insect, or mammalian cells or with cell-free extracts.

Cell-free expression of membrane proteins

Cell-free expression generates proteins by coupled or successive transcription and translation in cell-free extracts of prokaryotic or eukaryotic cells. Compared to conventional in vivo expression, cell-free in vitro protein synthesis procedures are extremely rapid, making them highly suited for screening expression templates and conditions. The advantages of cell-free expression systems include the possibility of producing proteins that are toxic to the host cell or have modified or isotope-labeled amino acids. Moreover, cell-free expression systems ensure a high protein yield per unit volume and the flexibility to adapt reaction conditions to the requirements of the synthesized protein (for example, the addition of cofactors).

Proteins produced by cell-free expression can be used for the same wide variety of downstream applications as in vivo-produced proteins, including activity assays, structural and functional analyses, protein–protein interaction studies, and the expression and analysis of open reading frames.

EasyXpress® Insect Kit II

For successful expression of membrane proteins

The EasyXpress Insect Kit II, derived from *Spodoptera frugiperda* insect cell extracts, is a system for cell-free protein expression. It contains the complete machinery for protein synthesis, including microsomes originating from the endoplasmic reticulum that are important for the successful synthesis of membrane proteins (Figure1).

- Complete homogenous system for highly reproducible results
- No additives required for expression of membrane proteins
- Membrane protein synthesis in a matter of hours
- Synthesis of posttranslational modification (gylcosylation, phosphorylation, and signal peptide cleavage)

Alternatively, EasyXpress *E.coli* Kits can be used for membrane protein expression, if posttranslational modifications are not required (1–3).

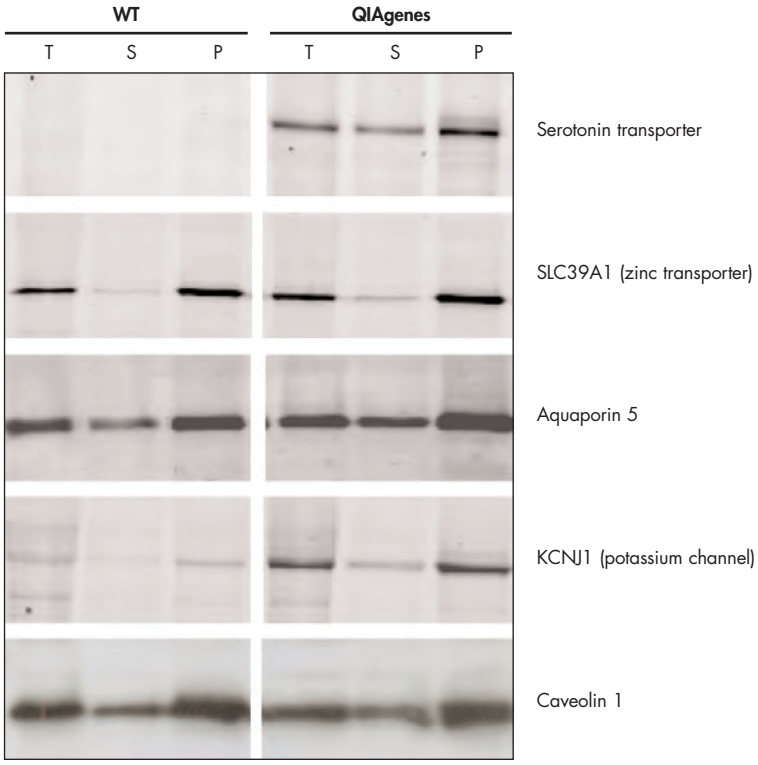


Figure 1. Expression of optimized QIAgenes expression constructs and wild-type (WT) coding sequences of the indicated membrane proteins using the EasyXpress Insect Kit II. Proteins were expressed in cell-free extracts. Expression levels were visualized after separation of crude lysates (**T**), supernatant (**S**), and pellet fraction (**P**) by SDS-PAGE and western blotting using Penta·His antibodies. Note that microsomes with integrated membrane proteins are visualized in the pellet fraction (**P**) after centrifugation of the reaction.

Optimized solution for membrane protein purification

Once expressed, the isolation of membrane proteins becomes the main challenge. In contrast to soluble proteins, the hydrophobic part of membrane proteins makes them difficult to solubilize as it results in the formation of aggregates. These can be solubilized using an appropriate detergent. Detergents are polar molecules (with hydrophobic and hydrophilic parts) and adhere to the hydrophobic part of a membrane protein, thus solubilizing it from the membrane. The solubilization results in a water soluble protein–detergent complex that can be isolated and subsequently purified. The selection of an effective detergent can be crucial for effective solubilization and purification of membrane proteins.

QIAGEN offers the innovative Ni-NTA Membrane Protein Kit which enables efficient solubilization and purification of His-tagged membrane proteins from prokaryotic or eukaryotic cell cultures or from cell-free expression systems.

Ni-NTA Membrane Protein Kit

For standardized solubilization and purification of membrane proteins

- Complete kit with 7 detergents, buffers, and Ni-NTA for purification
- Standardized procedure for handling membrane proteins
- Easy-to-follow protocols eliminate tedious evaluation and result in time savings
- Starting material is *E.coli*, insect cell culture, or cell-free extracts

The Ni-NTA Membrane Protein Kit is the first standardized method for detergent screening and purification of membrane proteins. This kit enables easy identification of the detergent most suitable for membrane protein solubilization (Figure 2) by allowing 5 screenings of each of 7 detergents. Once the optimal detergent is identified, the kit can be used for 5 affinity purification procedures (Figure 3).

The complete procedure can be reproducibly scaled up. Detergents are available in bulk for large-scale experiments.

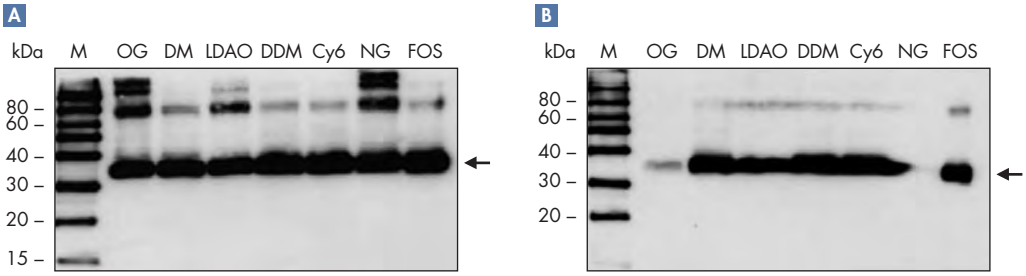


Figure 2. Purifying a bacterial membrane protein from bacterial cultures — screening for the optimal detergent. *E. coli* cells overexpressing the 35 kDa membrane protein NhaA were pelleted and lysed. The cell membranes were resuspended in the indicated detergents (included in the Ni-NTA Membrane Protein Kit). Aliquots of the total protein fraction were used for SDS-PAGE and western analysis. After centrifugation of the mixture, aliquots of the soluble membrane fraction were taken for SDS-PAGE and western analysis. A Western blot of total protein fraction, and B western blot of detergent-solubilized membrane protein fraction. NhaA protein is visualized by immunodetection of the His tag (arrows). As shown in B, detergents DM, LDA0, DDM Cy6, and FOS are suitable for solubilization of NhaA. **M**: His-tagged marker proteins.

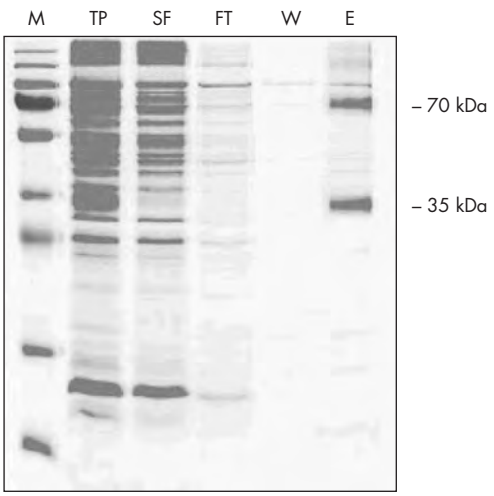


Figure 3. Purification of a membrane protein using Ni-NTA. NhaA was purified on Ni-NTA Superflow using buffers containing DDM. Fractions were separated on an SDS-PAGE gel and proteins visualized by Coomassie® staining. NhaA can be visualized in the stained gel in both its monomeric and dimeric forms (arrows). **TP**: total protein; **SF**: soluble fraction; **FT**: flow-through fraction; **W**: wash fraction; **E**: eluate; **M**: markers.

Innovative solutions for membrane protein crystallization

In addition to tedious expression and purification steps, crystallization of membrane proteins remains one of the greatest challenges in structural biology. Recent research (4) has proven that crystallization in meso phase (5) is the method of choice for membrane proteins. Meso phase mimics the lipid bilayer environment of the plasma membrane and provides close to natural conditions so that the membrane protein is folded correctly.

NeXtal® CubicPhase crystallization products

Current methods for the setup of *in meso* experiments include numerous manual steps. NeXtal CubicPhase crystallization products enable fully automatable, high-throughput membrane protein crystallization setup using a standard liquid-handling robot. While the standard approach for setting up an *in meso* experiment requires active mixing of lipids and the protein solution, the NeXtal CubicPhase procedure relies on passive diffusion of the protein within the lipids. Water evaporation and subsequent solution concentration, as well as the effect of the precipitant solution, drives a structural change of the lipid enabling protein crystallization (Figure 4).

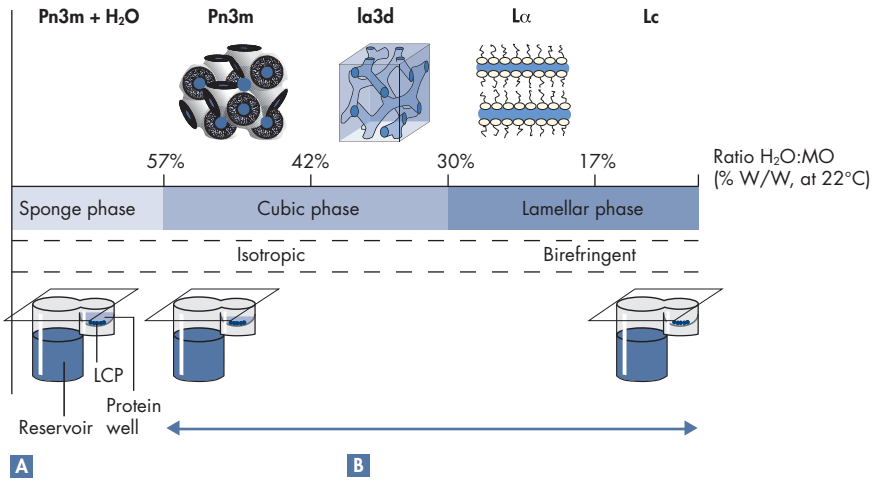


Figure 4. The NeXtal CubicPhase procedure. A Protein solution is dispensed onto dried lipids (monoolein). There is an excess of water when the experiment commences. This is known as the sponge phase. B Water evaporation triggers concentration of protein and precipitant and also alters the structure of the meso phase. Once an equilibrium in the water pressure at the top of the reservoir and the protein well is reached, the meso phase structure is stabilized in cubic phase or lamellar phase. Crystallization will eventually occur if the optimal level of supersaturation is reached and if the protein three-dimensional organization is correct. Adapted from Caffrey, 2008 (6).

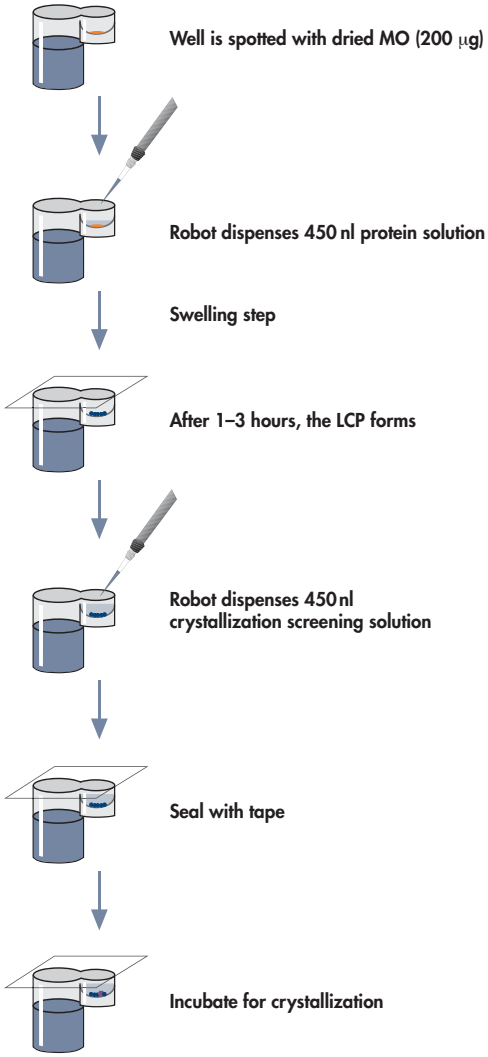


Figure 6. Workflow for setting up a crystallization experiment on the NeXtal CubicPhase µplate. The membrane protein solution is dispensed directly onto the dry lipid (monoolein). The protein solution diffuses into and rehydrates the lipid resulting in the passive formation of the lipidic meso phase.

The NeXtal CubicPhase Crystallization System includes:

- **NeXtal CubicPhase µplate**

  Extra airtight NeXtal Evolution µplates delivered prefilled with monoolein (MO) enable automated setup of the meso phase experiment upon hydration with the membrane protein solution (Figure 5). The innovative plate design provides optimal optical properties enabling visualization of transparent crystals. The semielliptical protein well makes crystal handling easier, while the larger rims ensure that virtually no evaporation occurs, resulting in highly reproducible experiments.

- **Dedicated screening solutions**

  A choice of 2 sets of 96 screening solutions optimized for *in meso* experiments are available. The CubicPhase I Suite contains buffered solutions with different added salts. The CubicPhase II Suite uses PEGs of differing molecular weight at set pH as precipitating agents.

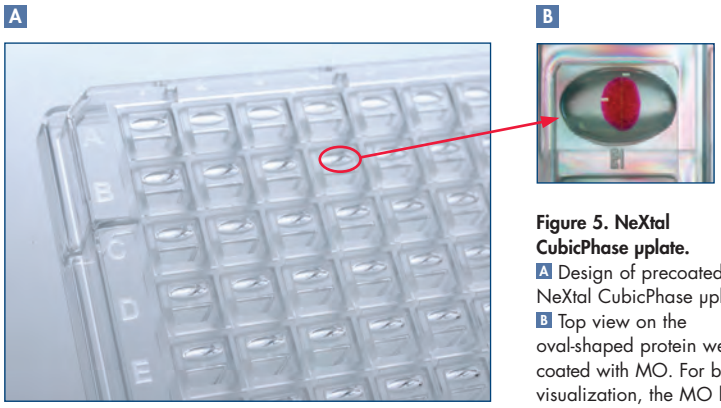


Figure 5. NeXtal CubicPhase µplate. A Design of precoated NeXtal CubicPhase µplate. B Top view on the oval-shaped protein well coated with MO. For better visualization, the MO has been colored with a red dye.

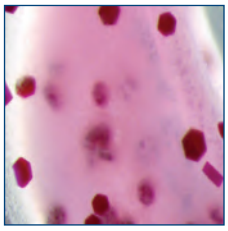


Figure 7. Crystal of bacterio-rhodopsin (BR) obtained with the NeXtal CubicPhase Cystallization System. The BR crystal diffracted at 1.5 Å and allowed confirmation of the structure that was previously solved.

The NeXtal CubicPhase procedure is easy to perform (Figure 6); it does not require mixing of lipids and the protein solution prior to dispensing. Only minute amounts of proteins (450 nl) are required compared to standard membrane protein crystallization setup. The innovative system provides an optimized solution for the generation of high-quality membrane protein crystals (Figure 7).

Express, purify, and crystallize — the complete solution

QIAGEN's protein technologies provide integrated, easy-to-use solutions from expression screening through to structural analysis, suitable even for challenging proteins such as those associated with the membrane. The EasyXpress System facilitates cell-free expression and successful synthesis of a range of membrane proteins of different origin and structure suitable for a multitude of downstream applications. Our dedicated Ni-NTA Membrane Protein Kit offers a standardized method for membrane protein solubilization and purification. Crystallization of membrane proteins can be easily achieved using the dedicated NeXtal CubicPhase product line.

Find out more about how our protein technologies can help streamline your research by visiting our protein resource page at www.qiagen.com/Products/ByApplication/Protein.

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2. Klammt, C., Schwarz, D., Fendler, K., Haase, W., Dötsch, V, and Bernhard, F. (2005). Evaluation of detergents for the soluble expression of ∝-helical and β- barrel-type integral membrane proteins by a preparative scale individual cell-free expression system. FEBS J. **272**, 6024.
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4. Cherezov, V. et al. (2007). High-Resolution Crystal Structure of an Engineered Human, 2-Adrenergic G Protein–Coupled Receptor. Science. **318**, 1258.
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Ordering Information

Product	Contents	Cat. no.
QIAgenes Expression Kit Insect/Mammalia	QIAgenes Expression Construct Insect/Mammalia, positive control, Penta·His Antibody, Ni-NTA Magnetic Agarose Beads	Varies
QIAgenes Expression Kit *E. coli*	QIAgenes Expression Construct *E. coli*, positive control, Penta·His Antibody, 4 Ni-NTA Spin Columns	Varies
EasyXpress Insect Kit II*	For 20 x 50 µl reactions: *Spodoptera frugiperda* insect cell extract, reaction buffers, in vitro transcription reaction components, RNase-Free Water, Gel-filtration Columns, and positive control DNA	32562

* Other kit sizes and/or formats available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
EasyXpress Protein Synthesis Kit (20)*	For 20 x 50 µl reactions: *E. coli* extract, reaction buffer, RNase-Free Water, and positive control DNA	32502
NeXtal CubicPhase Kit	2 x 96-well plates coated with monoolein and 2 deep-well blocks containing 96 x 1.5 ml solutions for crystallization of membrane proteins	130807
NeXtal CubicPhase µplate (10)	10 x 96-well plates coated with monoolein for crystallization of membrane proteins	130803
NeXtal CubicPhase µplate (100)	100 x 96-well plates coated with monoolein for crystallization of membrane proteins	130805
NeXtal DWB CubicPhase I Suite	96 x 1.5 ml solution for crystallization of membrane proteins	130928
NeXtal DWB CubicPhase II Suite	96 x 1.5 ml solution for crystallization of membrane proteins	130929
NeXtal Evolution µplate (10)*	10 x 96-well plates for protein crystallization	132045
Ni-NTA Superflow (25 ml)*	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
Ni-NTA Superflow Cartridges (5 x 1 ml)*	5 cartridges prefilled with 1 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30721
Ni-NTA Membrane Protein Kit	For 5 detergent screenings and 5 affinity purifications: 7 detergents, buffers, Ni-NTA Superflow, Penta·His Antibody, disposable columns	30610
Detergent DM	2 g n-Decyl-β-D-maltopyranoside (DM)	34114
Detergent DDM	2 g n-Dodecyl-β-maltoside (DDM)	34124
Detergent OG	5 g n-Octyl-β-D-glucopyranoside (OG)	34134
Detergent LDAO	2 g N,N-Dimethyldodecylamine-N-oxide (LDAO)	34144
Detergent NG	2 g n-Nonyl-β-D-glucopyranoside (NG)	34154
Detergent FOS-choline-16	1 g FOS-choline-16 (FOS)	34164
Detergent Cymal 6	2 g Cymal 6 (Cy6)	34174

* Other kit sizes and/or formats available; see www.qiagen.com.

All products listed above are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products.

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