

MembraneMax™ Protein Expression Kits

Maximum yield, solubility, and purity

More than 30% of the human genome is devoted to the production of membrane proteins, which play critical roles in cell-to-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, and transport. Because of their varied cellular functions, membrane proteins are ideal drug targets. However, membrane proteins as a class are often difficult to study; cell toxicity and inclusion bodies limit protein yield, and tedious optimization of conditions makes purification difficult and time-consuming.

Based on the MembraneMax™ reagent—a planar phospholipid membrane bilayer surrounded by a scaffold protein (also called a nanolipoprotein particle, or NLP)—the MembraneMax™ Protein Expression Kit overcomes these challenges, allowing you to produce high yields of soluble (dispersed) membrane pro-

teins. The MembraneMax™ *HN* Protein Expression Kit also incorporates His-tagged MembraneMax™ reagent for purification. Both kits deliver scalable, cell-free expression of nano- to milligram quantities of your membrane protein, and are amenable to high-throughput protein synthesis for screening applications, as well as the expression of toxic proteins. Key advantages include:

- Optimized, cell-free expression—high yields of membrane proteins from nanogram to milligram quantities
- MembraneMax™ reagent—enables a monodispersed population of soluble membrane protein–NLP complexes
- Convenient kit format—includes the necessary reagents for expression; simply add your gene of interest



Protein Expression

High yield for your downstream analysis

Expression of membrane protein is often very difficult due to insolubility in bacterial systems, *in vivo* toxicity, or simply low yield in mammalian systems. Using our Ultimate™ ORF collection as a source, we tested membrane proteins representing various sizes (8–51 kDa) and complexity (2–7 transmembrane domains). With the addition of the MembraneMax™ reagent, the majority of proteins were expressed at >0.1 mg/ml of protein (Table 1), a concentration sufficient and suitable for most downstream applications (Table 2).

Enhanced protein solubility

One of the most pervasive problems in protein expression is the insolubility of recombinant proteins, often seen as inclusion bodies. This is especially acute when working with membrane proteins that have both hydrophilic and hydrophobic domains as well as complex folding motifs. MembraneMax™ Protein Expression Kits use the novel MembraneMax™ reagent to enhance the solubility of overexpressed membrane proteins of a wide range of sizes and complexities (Figure 1).

Simple purification method

There are many purification challenges that have notoriously impeded research on membrane proteins, such as tedious and time-consuming testing of various detergents and extraction conditions in order to separate out other endogenous membrane proteins while maintaining the stability and function of your protein. The MembraneMax™ *HN* kit contains His-tagged MembraneMax™ reagent, which simplifies purification of the membrane protein–NLP complex without having to add a tag to your membrane protein. You also have the flexibility to design

your DNA template with your favorite purification tag at the N- or C-terminus of your membrane protein, which when used with the MembraneMax™ *HN* module enables tandem purification for very clean membrane protein–NLP complexes. Whether you choose to work with a native membrane protein or a tagged version, our MembraneMax™ kits (Table 3) give you the flexibility you need.

Table 1—High yields achieved using the MembraneMax™ system. A small sample of proteins that we have expressed using the MembraneMax™ Protein Expression Kit; 64% of the proteins analyzed were expressed at >0.1 mg/ml of reaction (n = 32).

Membrane protein (GenBank accession number)	Yield (mg/ml)
NM_002102.2	0.41
J02755.1	0.33
NM_004382	0.30
NM_002413.3	0.30
NM_002102.2	0.30
NM_004528.2	0.30
NM_001432.1	0.30
NM_000024.3	0.30
NM_181640.1	0.30
BC025781.1	0.30
BC001788.1	0.30
NM_000739.2	0.30
NM_000738.2	0.27
NM_032483.2	0.20

Table 2—Downstream applications for analysis of membrane proteins.

Antibody production
Crystallography
Immunoprecipitation
Ligand binding or functional assays
Mass spectrometry
Nuclear magnetic resonance (NMR)
Protein array construction

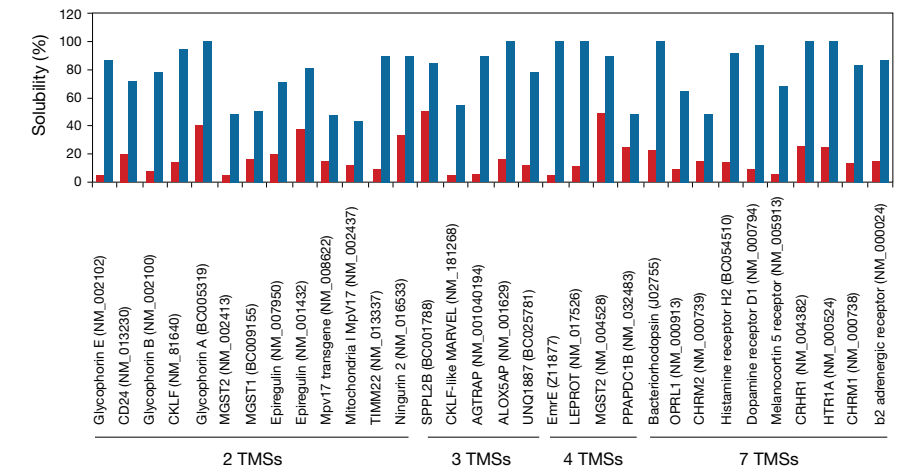


Figure 1—Soluble membrane protein expression achieved using the MembraneMax™ *HN* kit. The *in vitro* expression and solubility of membrane proteins of different topologies, sizes, origins, and proposed roles were analyzed. Proteins were expressed in the presence (blue) or absence (red) of MembraneMax™ *HN* reagent. For the analyzed data set, the overall solubility increased from $17.3 \pm 2.2\%$ (in the absence of NLPs) to $78.8 \pm 3.4\%$ (in the presence of NLPs). GPCRs exhibited a remarkable increase in solubility in the presence of NLPs. TMS = transmembrane segments.

Scalable and easy to use

Simply combine your T7 *E. coli* expression vector encoding your membrane protein with Expressway™ cell-free *E. coli* extract and MembraneMax™ reagent (both included in the kit), incubate for 2 hours, and purify (Figure 2). Within a day, you can generate milligrams of soluble membrane protein for downstream analysis, without detergents or tedious reconstitution protocols. The cell-free format enables high-throughput expression screening of multiple samples, or large-scale production of a single membrane protein.

Visit www.invitrogen.com/membranemax to find out more about our innovative MembraneMax™ products and to place your order today.

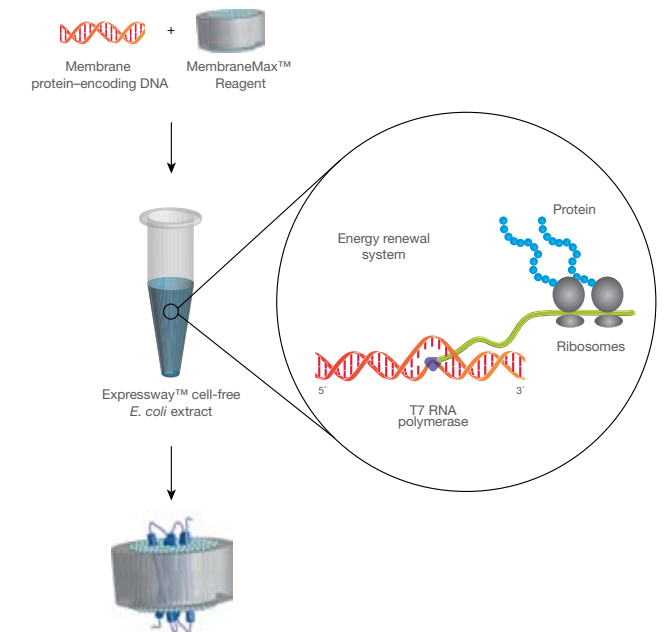


Figure 2—One-day protocol for membrane protein expression and purification. MembraneMax™ Protein Expression Kits are simple to use—just add your gene of interest. The kits contain all of the necessary components to produce high yields of soluble membrane proteins, using a simple and scalable method.

Table 3—Select the best MembraneMax™ kit for your research.

Kit	Features	20 rxns	100 rxns
MembraneMax™ Protein Expression Kit	<ul style="list-style-type: none"> • Microgram to milligram yield • Soluble, dispersed protein for downstream analysis • Convenient kit format • Includes native MembraneMax™ reagent 	A10632	A10633
MembraneMax™ <i>HN</i> Protein Expression Kit	<ul style="list-style-type: none"> • Microgram to milligram yield • Soluble, dispersed protein for downstream analysis • Convenient kit format • His-tagged MembraneMax™ reagent for purification of native membrane protein 	A10634	A10635



Protein Expression