

MembraneMax™ Protein Expression Kits

Catalog nos. A10632, A10633, A10634, A10635

Part no. A10608; Rev. Date: 29 May 2008

QUICK
REFERENCE
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Instructions for successfully using the MembraneMax™ Protein Expression Kits (MembraneMax™ and MembraneMax™ *HN*) to synthesize your recombinant membrane protein of interest from an expression construct are given below. For detailed instructions, refer to the manual supplied with the kit (Part no. A10604; also available at www.invitrogen.com).

Using the Correct MembraneMax™ Kit

MembraneMax™ Protein Expression Kit: Use this kit if you wish to express your membrane protein as a fusion protein containing an N- or C-terminal polyhistidine tag (6xHis) or any other tag.

MembraneMax™ *HN* Protein Expression Kit: Use this kit if you wish to express your membrane protein in its native state (i.e., untagged) or if you wish to use a tag other than 6xHis.

Note: MembraneMax™ *HN* Reagent contains a 6xHis tag (*HN* = His-tagged *NLP*; *NLP* = nanolipoprotein particle), therefore you **cannot** use this to express fusion proteins tagged with 6xHis. However, if your fusion protein contains an affinity tag other than 6xHis **and** you have used the MembraneMax™ *HN* Protein Expression Kit to synthesize it, you can utilize tandem affinity purification to achieve protein-NLP complex purity approaching 100%.

Experimental Outline

1. Generate DNA template (for detailed guidelines, refer to the manual supplied with the kit).
2. Purify your DNA template.
3. Perform the membrane protein synthesis reaction (see reverse for the detailed protocol).
4. Analyze recombinant membrane protein by polyacrylamide gel electrophoresis or other method of choice.
5. Purify your recombinant protein, if desired.

Generating the DNA Template

T7-based pEXP1-DEST, pEXP3-DEST, pEXP4-DEST, pEXP5-NT/TOPO®, and pEXP5-CT/TOPO® vectors that are available from Invitrogen are ideally suited for use with MembraneMax™ Protein Expression Kits to generate the DNA template. However, other expression vectors and DNA templates (including linear DNA and PCR products) may be used. For proper expression, all templates must contain the T7 promoter, an initiation codon, and RBS upstream of the gene of interest, and a stop codon and T7 terminator sequence downstream of the gene of interest. The figure below depicts the required regulatory elements in their optimal configuration for enhanced protein yield.



Once the DNA template is generated, purify the DNA before proceeding to the protein synthesis reaction. **Do not gel-purify** your DNA, as purified DNA solution obtained from agarose gels inhibits protein synthesis reaction. For rapid isolation of high quality templates from plasmid DNA and PCR products, we recommend PureLink™ HiPure Plasmid and PureLink™ PCR Purification Kits, respectively. For more information on purifying your DNA template, including alternative methods of purification, refer to the the manual supplied with the kit or available at www.invitrogen.com.

Materials Needed for the MembraneMax™ Protein Synthesis Reaction

Supplied by the user:

- Expression construct or other suitable DNA template (purified; resuspended in TE or sterile deionized water at >500 ng/μl)
- For each reaction, sterile, RNase-free 1.5 ml microcentrifuge tubes or other suitable reaction vessels
- RNase-free pipette tips and microcentrifuge tubes
- Thermomixer (set to 30°C or 37°C and 1,200 rpm)
- Optional: Standard shaking incubator (set to 300 rpm; instead of thermomixer)
- Optional: Labeled methionine (if producing labeled recombinant protein)

Supplied with the kit:

- MembraneMax™ or MembraneMax™ *HN* Reagent
 - Expressway™ *E. coli slyD*⁻ Extract; thaw on ice
 - Expressway™ 2.5X IVPS Reaction Buffer (–amino acids); thaw on ice
 - Expressway™ 2X IVPS Feed Buffer; thaw on ice
 - T7 Enzyme Mix; keep on ice and store at –20°C after initial use
 - 50 mM Amino Acids (–Met)
 - 75 mM Methionine
 - DNase/RNase-Free Water
 - Optional*: pEXP5-CT/bR control plasmid (0.5 μg/μl in TE Buffer, pH 8.0) and all-trans retinal (10 mM in DMSO)
- *The pEXP5-CT/bR control plasmid and all-trans retinal are provided for use in a rapid colorimetric MembraneMax™ control assay. For a detailed control assay protocol for the refer to the manual supplied with the kit or available at www.invitrogen.com.

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Performing the MembraneMax™ Protein Synthesis Reaction

Use the protocol below to synthesize your recombinant membrane protein from the DNA template. The amounts given are for a standard 100 µl reaction; if you are scaling up the reaction, adjust the volume of reagents accordingly.

1. For each sample, add the following reagents to the appropriate reaction vessel.

Reagent	Protein Synthesis Rxn	Positive Control Rxn	Negative Control Rxn
<i>E. coli slyD</i> ⁻ Extract	20 µl	20 µl	20 µl
2.5X IVPS Reaction Buffer (-amino acids)	20 µl	20 µl	20 µl
50 mM Amino Acids (-Met)	1.25 µl	1.25 µl	1.25 µl
75 mM Methionine	1 µl	1 µl	1 µl
MembraneMax™ or MembraneMax™ HN Reagent	2 µl	2 µl	-
T7 Enzyme Mix	1 µl	1 µl	1 µl
DNA Template	1 µg	-	-
pEXP5-CT/bR Control Plasmid	-	2 µl	2 µl
DNase/RNase-Free Water	To a final volume of 50 µl		

2. Close the tube and incubate sample in the thermomixer (1,200 rpm) at 30-37°C for 30 minutes.

3. During the 30 minute incubation, prepare the Feed Buffer. For each sample, add the following reagents to a sterile, RNase-free microcentrifuge tube. For multiple samples, you may scale up the volume of reagents used accordingly and prepare one master mix.

Reagent	Protein Synthesis Rxn	Positive Control Rxn	Negative Control Rxn
2X IVPS Feed Buffer	25 µl	25 µl	25 µl
50 mM Amino Acids (-Met)	1.25 µl	1.25 µl	1.25 µl
75 mM Methionine	1 µl	1 µl	1 µl
10 mM all-trans retinal	-	0.5 µl	0.5 µl
DNase/RNase-Free Water	To a final volume of 50 µl		

4. After 30 minutes of incubation (from Step 2 above), add 50 µl of the Feed Buffer to each sample (total volume = 100 µl).

5. Cap the tube and return the sample to the thermomixer (1,200 rpm). Incubate the sample at 30-37°C for 1 hour 30 minutes. If desired, you can increase incubation up to 4 hours.

6. Place the reaction on ice and analyze your sample using a method of choice. You can also store the sample at -20°C for future processing or analysis.

Note: To generate radiolabeled membrane protein using [³⁵S]-methionine, use 1 µl each of [³⁵S]-methionine and unlabeled 75 mM methionine when initiating the reaction, then again in the feed buffer.

To generate labeled protein using selenomethionine, use 2 µl of selenomethionine only, **do not** add unlabeled methionine.

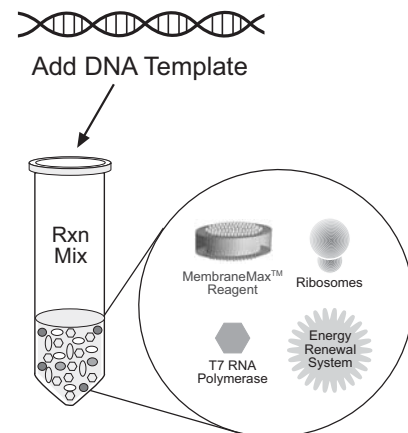
Analyzing Samples

Once you have performed the membrane protein synthesis reaction, you may use any method of choice to analyze your sample. Generally, the amount of membrane protein produced in a MembraneMax™ Protein Synthesis reaction is sufficient to allow detection on a Coomassie-stained protein gel, by western blot analysis, by enzymatic activity assay, by affinity purification, or by autoradiogram (if labeled with [³⁵S]-methionine).

Note: Expression levels may vary from protein to protein, and depends on the nature of the membrane protein and the configuration of the DNA template.

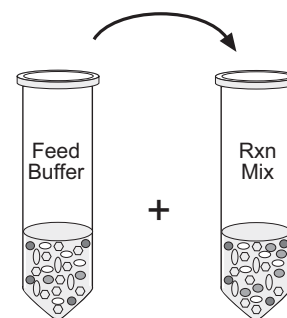
- If you plan to analyze your sample using polyacrylamide gel electrophoresis, first precipitate the proteins with acetone to remove background smearing.
- If you have performed trace labeling using [³⁵S]-methionine, you may use TCA precipitation to determine the amount of radiolabeled methionine incorporated and to calculate the protein yield.
- If you have expressed your recombinant membrane protein with a fusion tag, you may perform western blot analysis utilizing an antibody appropriate to the epitope tag.

For more information on acetone precipitation, general guidelines for gel electrophoresis, and calculating the yield of protein, refer to the manual supplied with the kit or available at www.invitrogen.com.



Incubate 30 min

Add Feed Buffer



Incubate 1 hr 30 min

Obtain Recombinant Membrane Protein

