The Expi293™ MembranePro™ Expression System for efficient, scalable production of MembranePro™ particles

The MembranePro™ Functional Protein Expression Kit provides users with an enriched sample of functional GPCRs and other cell-surface membrane proteins. This is accomplished through a less labor-intensive and easier workflow than used to prepare plasma membranes, the traditional source of these proteins, for pharmacokinetic and biochemical activity assays.

The efficiency, high cell density, and scalability of the Expi293™ Expression System make it ideally suited for the production of MembranePro™ particles in quantities suitable for a wide variety of low- or high-throughput applications. Compared to the standard adherent cell protocol used with the MembranePro™ kit, the protocol adapted for the Expi293™ MembranePro™ Expression System offers the following benefits:

- Efficiency—over 20-fold increase in membrane protein yield compared to the adherent culture systems
- Scalability—easy scale-up without the need for multiple flasks
- Ease of use—no enzymatic or mechanical dissociation required

Here we outline a protocol for adapting the MembranePro™ kit for use with the Expi293™ system. We compare the activity and yield from this new production method to that of the standard protocol described in the MembranePro™ manual, which uses adherent 293FT cells.

Materials

- Expi293™ MembranePro™ Expression System (Cat. No. A25869 and A25870)
- Expi293F™ Cells (Cat. No. A14257)
- pEF6/V5-His TOPO® TA Expression Kit (Cat. No. K961020)
- 125 mL culture flasks (Corning, Cat. No. 431143)
- CO_2-controlled, 37°C incubator containing a plate shaker

Methods

1. **Prepare cells:** Seed and maintain Expi293F™ cells as directed in the Expi293™ Expression System manual. To transfect cells on "day 0", seed the cells on the previous day (day –1) at a density of 2.0 x 10^6 viable cells/mL.
2. **Determine the number of cells you will need for your experiment:** On the day of transfection (day 0), determine cell viability using an automated cell counter or the trypan blue dye exclusion method. **Important:** Cells should not be clumping, and viability of cells must be over 95% to proceed with transfection. Dilute cells to 2.9 x 10^6 cells/mL with Expi293™ Expression Medium. You will need 26 mL of cells for a single 36 mL final culture.
3. **Prepare transfection complexes:** For each flask of cells to be transfected, prepare transfection complexes as follows:
   - Combine 9 µg of expression plasmid with 27 µg of MembranePro™ Reagent. Add this to 4 mL Opti-MEM® I Reduced Serum Medium and mix gently by pipetting up and down 4 times.
   - Gently mix the ExpiFectamine™ 293 Reagent by pipetting up and down. Dilute 180 µL of reagent in 4 mL Opti-MEM® I medium and mix gently by pipetting up and down 4 times.
   - Add the diluted DNA to the diluted ExpiFectamine™ 293 Reagent and mix by pipetting up and down 4 times.
   - Incubate the mixture for 20 minutes at room temperature to allow DNA–MembranePro™ Reagent–ExpiFectamine™ 293 Reagent complexes to form.
4. **Transfect cells:** Add the 8 mL of transfection complex mixture to a flask containing 26 mL of Expi293F™ cells. Place the flask on a shaker set to 125 rpm inside a humidified 37°C tissue culture incubator supplied with 8% CO_2 (see the Expi293™ Expression System manual for further culturing details).
5. **Add enhancers:** 18–24 hours post-transfection, in a tissue culture hood, add 150 µL of Enhancer 1 and 1.5 mL of Enhancer 2 to the flask of transfected cells. Return the flask to the incubator with shaking for an additional 48 hours. For convenience, you may prepare a cocktail of Enhancer 1 and 2 if you are adding enhancers to multiple flasks.
6. **Harvest and clarify supernatant:** Decant the culture into a 50 mL conical or Oak Ridge tube and centrifuge at 3,000–5,000 x *g* for 15 minutes to remove cell debris. Transfer the culture supernatant to a fresh tube, and repeat centrifugation to remove any contaminating cells or debris. This double centrifugation step is essential due to the high density of Expi293F™ cell cultures.
7. **Isolate MembranePro™ particles:** To precipitate MembranePro™ particles, follow the protocol in the MembranePro™ manual by adding 1/5 volume of the MembranePro™ Precipitation Mix to the cell culture supernatant and mixing by inverting 10 times. Incubate the sample overnight at 4°C. The next day, pellet the particles in a swinging bucket centrifuge (essential to collect the particles in a concentrated pellet at the bottom of the tube) at 3,000–5,000 x *g* for 15 minutes. Pelleted particles may or may not be clearly visible following centrifugation. Resuspend the particles in 1X PBS for use, or aliquot for storage at –80°C.

Results and discussion

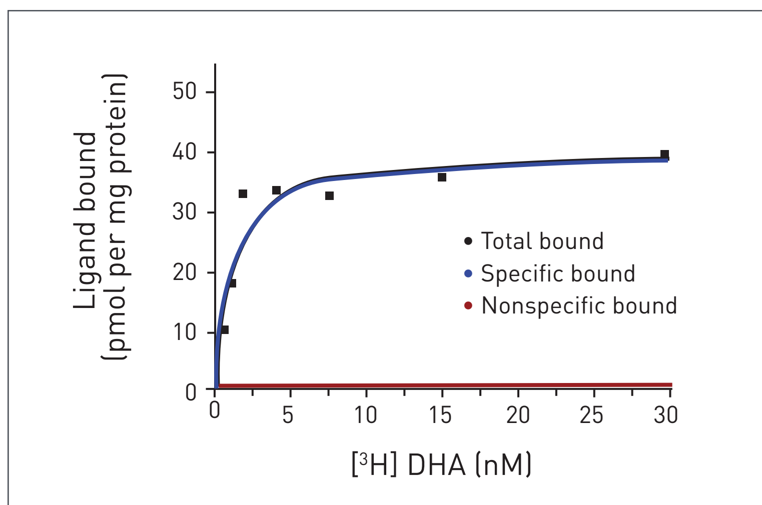
The protocol for producing MembranePro™ particles using the Expi293™ MembranePro™ Expression System yielded approximately 44-fold more product* than the standard protocol using adherent 293FT cells (Figure 1, top). Both protocols were performed using ~30 mL cultures. However, the higher cell density and efficiency of protein production in the Expi293™ MembranePro™ Expression System enabled a higher yield of 2.79 mg of product, compared to 0.063 mg for the standard adherent cell protocol.

To compare the quality of the two preparations, we examined them for B2Ar antagonist binding activity. Limiting amounts of MembranePro™ protein were challenged with [^{3}H] DHA, a B2Ar antagonist, in saturation binding experiments as shown in Figure 1. Both samples demonstrated similar pharmacologically appropriate K_d values for this ligand–receptor combination. Additionally, the similar B_{max} values indicate that the Expi293™ method maintained GPCR quality and efficiency of capture on the MembranePro™ particle surface despite the 44-fold increase* in production yield.

*Protein yield is dependent on the membrane protein being expressed.

Standard protocol

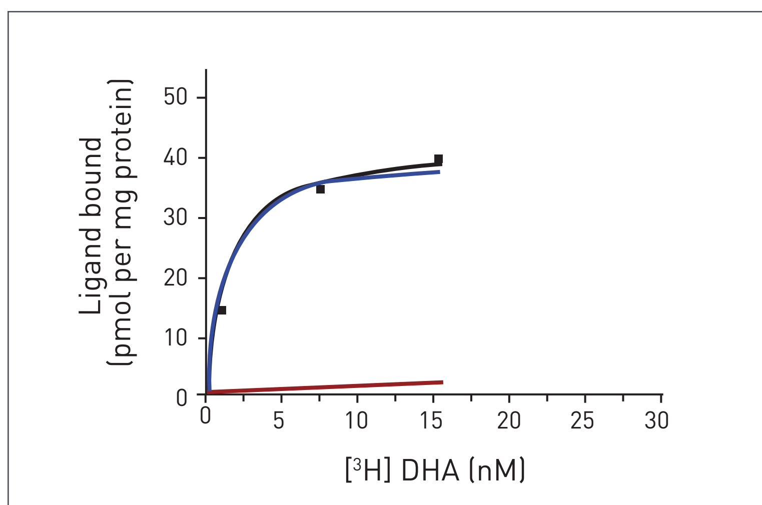
Protein yield: 1 mL at 0.063 µg/µL
0.64 µg protein used per binding reaction



	Specific bound
B_{max}	39.68 pmol ligand per mg protein
K_d	0.93 nM

Expi293™ MembranePro™ Expression System protocol

Protein yield: 1 mL at 2.79 µg/µL
0.5 µg protein used per binding reaction



	Specific bound
B_{max}	30.08 pmol ligand per mg protein
K_d	1.06 nM

Figure 1. Comparison of the standard protocol and Expi293™ MembranePro™ Expression System protocol for production of MembranePro™ particles.

Find out more at lifetechnologies.com/membranepro

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