Preparation of Rabbit Red Blood Cell Membranes for Transmembrane Pores

Rabbit blood is purchased from www.hemostat.com

1st Wash

1a. Take 60 ml raw rabbit blood and divide in 10 ml aliquots in 50 ml centrifuge polypropylene tubes.
1b. In each tube, add 40 ml MBSA buffer (150 mM NaCl, 10 mM Na-MOPS, 0.1 % Bovine Serum Albumine (1 mg/ml), titrated with NaOH to reach pH7.4, then filtered with Nalgene Filters (0.2 μm), and stored at 4 Celsius degrees).
1c. This 50 ml solution is centrifugated at 1780 rpm (700 g) for about 10 minutes (in swing bucket).
1d. Remove gently the supernatant, and re-suspend gently in MBSA (total volume of 50 ml) buffer again by reversing the 50 ml centrifuge tube upside down.
1e. Repeat steps 1c-1d for a total of 4 times.

At the end you expect a 3 ml packed Red blood Cell Membranes

2nd Wash

2a Extract packed RBCM. Transfer to new centrifugation tubes.
2b. Re-suspend the pellet in lysis buffer up to a final volume of 50 ml (5 mM Na_2HPO_4, 1mM EDTA, pH8.0)
2c Centrifugate at high speed centrifuge – 15000 (≥ 20,000 g) for 30 minutes

3rd Wash

3a. Collect soft pellet
3b. Transfer up to 50 ml centrifuge tubes (equivalent to maxi-prep tubes)
3c. Wash the pellet in NaH_2PO_4 until no pink color remain for 10-20 minutes
3d. Repeat this procedure about 5 times until no pink color remain

4th Aliquot and freeze - 150 μl aliquots.
RBLMs were quantified with DC Assay - **Membrane Protein Biorad Protocol**

For DC Assay and Rabbit Red Blood Cell Membranes, please take a look at Steve Cheley et al., Protein Eng. 1997 Dec;10(12):1433-43.