ATPase assay
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This protocol relates to ATPase activity measurements presented in Wang et al. 2020. It is based on an NADH-coupled ATPase assay (Scharschmidt et al., 1979). Please address concerns to Jesper Levring (jlevring@rockefeller.edu).

Reagents
- Purified bMRP1
- 1 M Tris-HCl, pH 8.0
- 2 M KCl
- Dithiothreitol (DTT)
- 0.075 % (w/v) digitonin. Solubilised stirring 12 hours at 4 °C, and filtered with a 0.22 µm filter.
- 50 mM NADH
- Phosphoenolpyruvate
- 10 mg/ml Pyruvate Kinase (Roche)
- 5 mg/ml Lactate Dehydrogenase (Roche)
- 10x ATP-Mg$^{2+}$ stocks, pH 8.0 (with KOH) at 0.5, 1, 2.5, 5, 10, 20, 40, and 60 mM.

bMRP1 activity measurement
1. Prepare reaction buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM KCl, 2 mM MgCl$_2$, 2 mM DTT, 0.06 % (w/v) digitonin, 60 µg/ml pyruvate kinase (Roche), 32 µg/ml lactate dehydrogenase (Roche), 9 mM phosphoenolpyruvate, and 150 µM NADH.
2. Dilute bMRP1 to 800 nM in reaction buffer. Keep bMRP1 at 4 °C until initiating the reaction.
3. In parallel, prepare a buffer control without bMRP1.
4. For substrate stimulation experiments add 10 µM LTC4 to the reaction mix and incubate 15 min at 4 °C.
5. Aliquot 27 ul of reaction buffer with or without bMRP1 into wells of a Corning 384-well Black/Clear Flat Bottom Polystyrene NBS Microplate.
6. Add 3 ul ATP-Mg$^{2+}$ from 10x ATP-Mg$^{2+}$ stocks to initiate the reactions.
7. Follow NADH consumption at 30 °C by monitoring fluorescence with an Infinite M1000 microplate reader (Tecan). Fluorescence is measured at $\lambda_{ex} = 340$ nm and $\lambda_{em} = 445$ nm at 30 s intervals for 45 min (Figure A).

NADH standards
1. Prepare solutions of NADH in reaction buffer at 0, 25, 50, 75, 100, 125, 150, 175, and 200 µM.
2. Aliquot 30 µl of each into a Corning 384-well Black/Clear Flat Bottom Polystyrene NBS Microplate.
3. Measure fluorescence at $\lambda_{ex} = 340$ nm and $\lambda_{em} = 445$ nm at 30 °C with the Infinite M1000 microplate reader (Tecan).
4. Obtain a standard curve for NADH by plotting fluorescence as a function of NADH concentration (Figure B).

ATPase activity
1. Determine the slope of the fluorescence decay in the linear range of NADH consumption. Correct for background NADH oxidation using the rate of decay in the sample without bMRP1.
2. Convert the fluorescence slope to the rate of NADH oxidation using the NADH standard curve.
3. Correct for bMRP1 concentration to calculate the rate of ATP turnover per molecule of bMRP1 (Figure C).

A. NADH consumption over time in samples with or without bMRP1. Regression is performed in the linear range of NADH consumption.
B. NADH standard curve.
C. Sample ATPase activity measurements of wild-type bMRP1 with or without 10 µM LTC₄.
