

Antibody Information						
Source	Antibody	Pab/Mab	Form	Antigen	Epitope mapped	Conditions tested
Sorscher	2-39.14	Mab mouse	Protein G column purified from tissue culture supernatant of hybridoma cells (Bovine Ig depleted from serum prior to use) 1 mg/ml (in PBS)	Human NBD2	1371-EPSAHLDPVTYQIIR-1385	*Western blot 1:1000 ~2000 (detailed protocol below) **IP

*Western Blot protocol

1. Total cell lysates prepared in RIPA (150mM NaCl, 1% IGEPAL CA-630, 0.5%Na-deoxycholate, 0.1% SDS, 50mM Tris-Cl, pH8.0) with protease inhibitor (Complete Mini, Roche, #11836153001).
2. Generally 20 µg total cell protein was sufficient to detect strong CFTR signal.
3. Cells used to detect CFTR: CFBE-wt CFTR, FRT, HEK 293 and HeLa cells transfected with various CFTR constructs.
4. Wash buffer: PBS with 0.1 % Tween
5. Blocking solution: 5% Dry milk in PBS-0.1% Tween
6. Antibody dilution used: 1:500 in blocking solution, overnight, 4 °C
7. Secondary antibody used: Goat anti-mouse HRP (Dako, cat no. P0447), 1: 5,000, 1 hour at room temperature
8. Thermo Pierce SuperSignal West Femto Maximum Sensitivity Substrate (#34095)
9. Kodak Biomax MR film used to develop image

**Immunoprecipitation protocol

1. Cell lysates prepared in lysis buffer (200 mM NaCl, 50 mM Tris, pH 7.5, 1% IGEPAL CA-630) with protease inhibitors (Complete Mini, Roche, #11836153001). Lysis: 10 min on ice, then spin at 14K rpm for 5 min. to remove nuclei and debris.
2. 500 mg total protein used per IP (from 293 cells transiently expressing NBD1 or CFTR)
3. Suggested amount of antibody per IP: 1-5µg diluted in some lysis buffer (~200-500µL)
4. Capture Ab on Dynabeads protein G (Invitrogen, cat# 100-03D) by 1 hour incubation at 4 °C with rotation, wash beads once with lysis buffer
5. Incubate cell lysate (volume about 200-250µL) with immobilized Ab for 1 hour at 4 °C, with rotation.
6. 3-5 washes with lysis buffer; finally add 5X sample loading buffer.

