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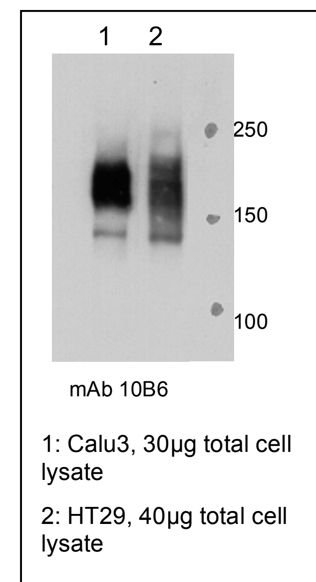
Antibody	Pab/Mab	Form	Antigen	Epitope mapped	Conditions tested
10B6.2	Mab mouse	Protein G column purified from tissue culture supernatant of hybridoma cells (Bovine Ig depleted from serum prior to use) 1 mg/ml (20% glycerol, 0.1% BSA, 0.01% Na-azide in PBS)	Human NBD1	Including residues WEEGFGELFE	*Western blot 1:500 (detailed protocol below) **IP IF
5A6.3	Mab mouse	Protein G column purified from tissue culture supernatant of hybridoma cells (Bovine Ig depleted from serum prior to use) 1 mg/ml (20% glycerol, 0.1% BSA, 0.01% Na-azide in PBS)	Human NBD1	No information. We suspect that the epitope may be non linear and there could be some measure of conformational specificity.	Very weak on western blot **IP IF: not tested

*Western Blot protocol for 10B6.2 antibody

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: HT29, Calu3, CFBE-wt CFTR, CFBE-ΔF CFTR, 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



mAb 10B6

1: Calu3, 30µg total cell lysate

2: HT29, 40µg total cell lysate

*Alternative Western Blot protocol

1. Cell lysates prepared in lysis buffer (200 mM NaCl, 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 15% glycerol, 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. 50 mg of a 293 cell lysate loaded on SDS gel for a very strong signal.
3. Blocking: 5% skim milk in TBS-N (0.5% NP-40/IGEPAL in TBS) for 40 min. at RT
4. Primary antibody (10B6.2): 1:1000 (equals 1mg/mL) in TBS-N for 1 hour at RT
5. wash buffer: TBS-N
6. Secondary Ab: Goat anti-mouse HRP (Dako, cat no. P0447), 1: 10,000, 40 min. at RT, in TBS-N
7. ECL reagents from GE Health Care (Amersham Biosciences)

8.` exposure to Kodak BioMax MR film

**<u>Immunoprecipitation protocol</u>

1. Cell lysates prepared in lysis buffer (200 mM NaCl, 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 15% glycerol, 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 protein G sepharose 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.