Western Blots Anti - P-Rex1 with mAb 6F12

(Heidi Welch lab protocol, October 2008)
Any questions: e-mail heidi.welch@bbsrc.ac.uk

- Wet transfer protein gel onto Immobilon-P (Millipore, PVDF membrane)

- Block #1: TBS-Tween + 5% dry non-fat milk (do not re-adjust pH), over night, 4°C, shaking

- Block #2: TBS-Tween + 1% BSA, 20 min, RT, shaking

- 1st AB: Mouse mAb culture supernatant anti-human P-Rex1, clone 6F12 at 1 : 10 in block solution #2 + 0.01% azide (can be stored at 4°C and re-used several times), 2 hrs, RT, shaking

- Washes: TBS-Tween, 3 rinses + 6 x 10 min, RT, shaking

- 2nd AB: BioRad rabbit anti mouse-IgG - HRP at 1:3000 in TBS-Tween, 1h, RT, shaking

- Washes: TBS-Tween, 3 rinses + 6 x 5 min, RT, shaking, followed by 2 x 5 min in TBS

- ECL (Amersham)

Recipes

TBS : 20 mM Tris (made from 1M stock at pH 8.0, RT), 150 mM NaCl

TBS-Tween : TBS + 0.05% Tween 20

Mouse monoclonal anti-P-Rex1 antibody 6F12
Clone 6F12, an IgG1 subtype, was raised by Marcus Thelen's lab (Bellinzona, Switzerland) against amino acids 1341-1375 of human P-Rex1 using standard techniques, and purified from hybridoma culture supernatants using γ-bind Sepharose. It was first described in: Welch HC et al, (2005), P-Rex1 regulates neutrophil function. Curr Biol. 15, 1867-1873.