University of Dundee

Contact:- Dr C. James Hastie

Antibody Datasheet

Product description:- Anti-WNK1

Antigen:- QNFNISNLQKSISNPPGSNLRTT
[Residues 2360 – 2382 of human]

Sheep Number:- S62B

Bleed Number:- First Bleed

Concentration:- 0.34 mg/ml

Formulation:- Phosphate Buffered Saline

Storage temperature:- –20 °C

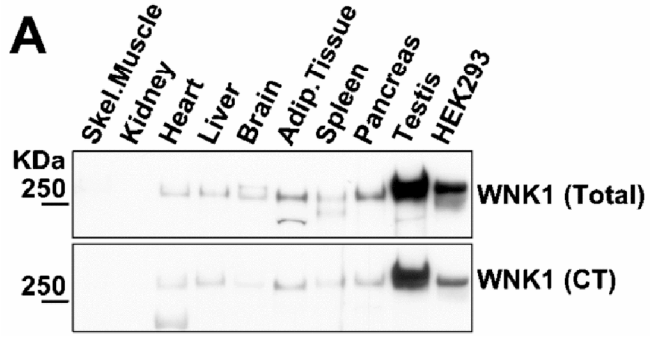
Purification Method:- Affinity purified against WNK1 (2360 – 2382) peptide

Working Concentration:-
Immunoblotting: 1 μg/ml for immuno blotting
Immunoprecipitation: 1 μg of antibody per 1 mg of cellular extract

Publication Reference:-

"The WNK1 and WNK4 protein kinases that are mutated in Gordon's hypertension syndrome phosphorylate and activate SPAK and OSR1 protein kinase"
Vitari, AC, Deak, M, Morrice, NA and Alessi, DR.
Biochem J, (2005). 391, 17 – 24.

Immunoblotting:

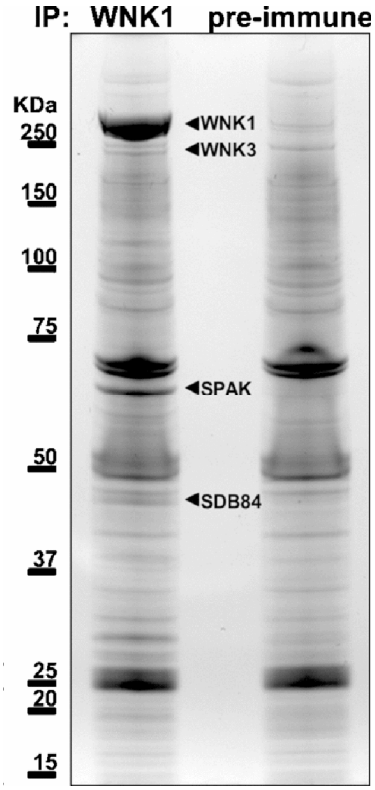


Rat tissue extracts (40 μg of protein) and HEK 293 cellular extract (40 μg of protein) were subjected to SDS-Page analysis before being transferred to nitrocellulose membrane and immunoblotted with anti-WNK1 at 1 μg/ml.

Binding of the primary antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).

Immunoprecipitation:

B



WNK1 and pre immune IgG antibodies were covalently coupled to protein G Sepharose in a ratio of 1 mg of antibody to 1 ml of resin using a dimethyl pimelimidate cross-linking procedure. As a pre-clearing step, 50 mg of rat testis lysate was incubated at 4 °C for 20 mins on a rolling shaker with 0.5 ml of protein G-Sepharose. The supernatant was then incubated at 4 °C for 1 hour on a rolling shaker with 50 μl of WNK1 or IgG-Protein G-Sepharose conjugated antibodies. The immunoprecipitates were washed four times with 10 ml of 10 mM Tris-HCl pH 8 / 0.1 mM EGTA. The resin was resuspended in SDS-Page sample buffer and subjected to SDS-Page analysis with staining with Coomassie blue.