University of Dundee

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Antibody Datasheet

Product description:- Anti-CRTC3 phospho Ser 329

Antigen:- GLQSSRS*NPSIQ [residues 323 - 334 of human]

Sheep Number:- S256D

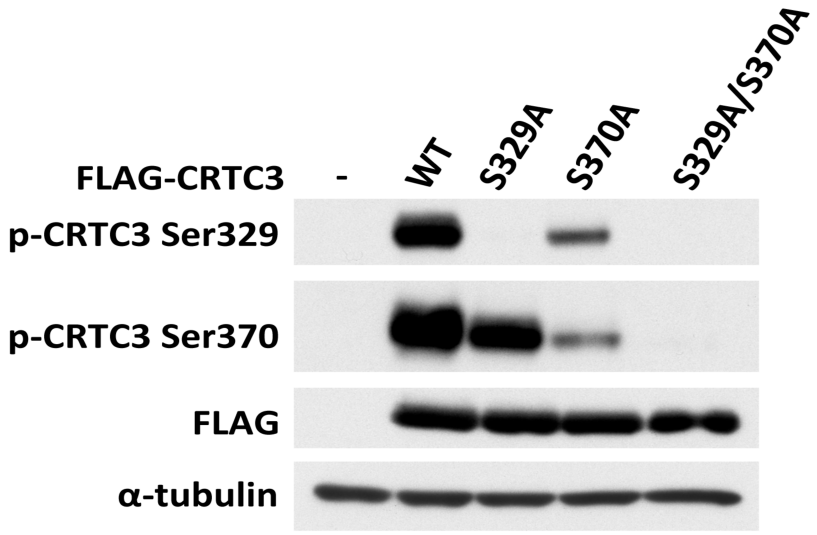
Formulation:- Phosphate Buffered Saline

Storage temperature:- –20 °C

Purification Method:- Affinity purified against phospho specific peptide

Working Concentration:- For immunoblotting use at 1 ug / ml, in the presence of 10 ug/ml of the non-phospho peptide

Immunoblotting:-
Detects recombinant wildtype CRTC3, but does not recognizes CRTC3 mutated at Ser 329 and does not recognize CRTC3 mutated at both Ser 329 and Ser 370. Note that the sequences surrounding Ser 320 and Ser 370 are very similar leading to some cross-reactivity.



Method: Constructs encoding wild-type Flag-CRTC3 and Flag-CRTC3 with relevant phosphosites mutated to Ala were expressed in HEK293 cells. Cell extract protein (25 ug) was separated by SDS-PAGE and immunoblotted with anti-phosphoSer329 (S256D) or anti-phosphoSer370 (S253D) at 1 ug/ml in the presence of 10 ug/ml of non-phosphorylated peptide antigen. Expression of each CRTC3 construct was verified by blotting using anti-FLAG.

Result: Antibodies raised against phosphoSer329 and phosphoSer370 detect overexpressed wild-type CRTC3 by immunoblot. The antibody against phosphoSer329 failed to detect CRTC3 with Ser329 mutated to Ala. The antibody against phosphoSer370 weakly detected CRTC3 when Ser370 was mutated to Ala but did not recognize CRTC3 with Ser329 and Ser370 mutated to Ala. Note that the weak cross-reactivity of the antibodies is due to the similarity in the sequences surrounding Ser329 and Ser370 in CRTC3.