University of Dundee

Contact:- Dr C. James Hastie

Antibody Datasheet

Product description:- Anti-CDKL5 (350 – 650)

Antigen:- GST-CDKL5 (350 – 650) [DU 50406]

Sheep Number:- S957D

Formulation:- Phosphate Buffered Saline

Storage temperature:- -20 °C

Purification Method:- Affinity purified against recombinant protein MBP-CDKL5

(350 - 650) [DU 35039]

Working Concentration:-

For immunoblotting use 0.5 - 1 ug/ml 3rd bleed

For immunoprecipitation use 1 ug antibody per 1 mg of cell extract

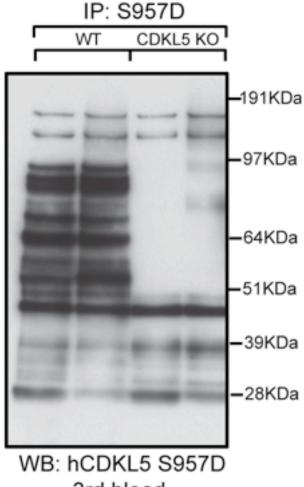
Immunoblotting:

Tested antibody for immunoblotting against Hela, U2OS and HAP-1 cell extracts, but many unspecific bands. Recommend running CDKL5 knockout (KO) extract in parallel. It does recognize CDKL5 in lysates, but recognizes a protein running slightly faster than CDKL5 non-specifically.

Concentration used: 0.5-1 ug / mL (1 hr at room temperature). Do not probe overnight at 4 degrees – very dirty, including bands at same size as target protein.

Immunoprecipitation followed by Immunoblotting:

human HAP1 cells



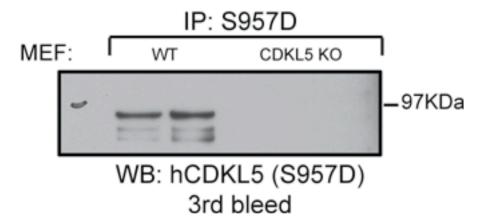
3rd bleed

Immunoprecipitation using the human CDKL5 (S957D) antibody. 2 milligrams of cell lysate from either wild type (WT) or CDKL5 knockout human HAP1 cells generated by CRISPR/CAS9-mediated genome editing were subjected to immunoprecipitation with 2 micrograms of CDKL5 antibody. Samples were resolved in a 4-12% Bis-Tris gel and incubated with the 0.5 micrograms/ml of antibody for 1 hour at room temperature. Secondary proteinG-HRP (Abcam, ab7460) was incubated 1:5000 for 1h at room temperature.

Please note:

In U2OS cells, a no-specific band at same Mw as CDKL5 is recognized – be careful. Unclear what this band is but appears in CDKL5 KO U2OS!!!

mouse embryonic fibroblasts



Immunoprecipitation from MEF extracts using the human CDKL5 (S957D) antibody. 1 milligram of cell lysate from either wild type (WT) or two CDKL5 KO mouse embryonic fibroblasts (MEF) were subjected to immunoprecipitation with 2 micrograms of the anti-human CDKL5 human antibody (S957D). Samples were resolved in a 4-12% Bis-Tris gel and incubated with the 0.5 micrograms/ml of antibody for 1 hour at room temperature. Secondary proteinG-HRP (Abcam, ab7460) was incubated 1:2500 for 1h at room temperature.