

PIM2 | Validation File

TARGET Pim-2 Proto-Oncogene, Serine/Threonine Kinase

CLONE NAME CHU61B

DESCRIPTION rat monoclonal

ANTIGEN USED PIM2 HIS-PIM2 recombinant protein

ISOTYPE IgG2a

SPECIES REACTIVITY human

LOCALIZATION Nuclear and cytoplasmic

POSITIVE CONTROL Tonsil

STORAGE BUFFER Tissue culture supernatant: 0.02% sodium azide

Purified antibody: PBS plus 1%BSA and 0.02% sodium azide. MAb concentration: 1mg/ml

STORAGE Aliquot and store at 4C. Do not freeze

 Recommended

 Inconclusive

 Not Recommended

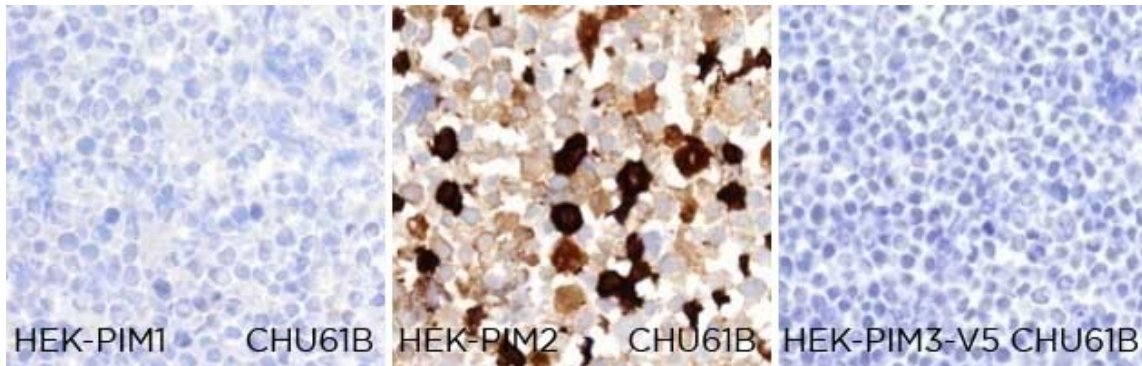
 Not Tested

APPLICATIONS

● | ICC | Immunocytochemistry

CHU61B is able to detect human PIM2 protein in immunocytochemistry

To confirm that CHU61B mAb recognizes human PIM2 protein, immunocytochemistry on frozen cytospin preparations PIM2, PIM1 and PIM3-V5 expressed in HEK293T cell line was performed.



● | WB | **Western Blotting**

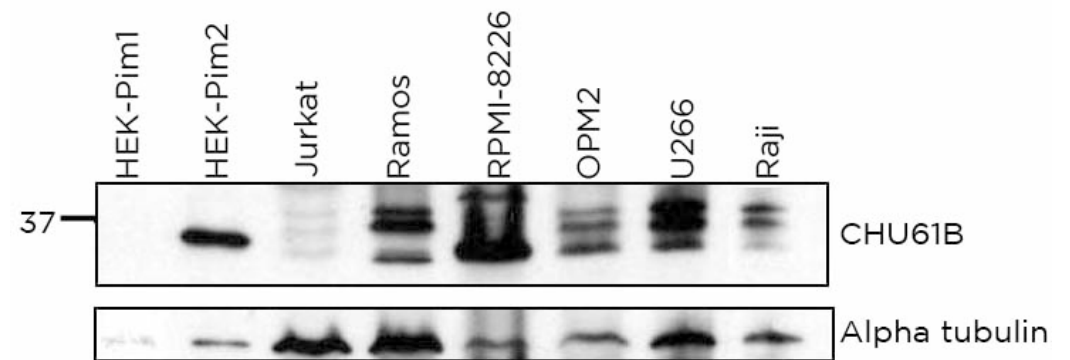
CHU61B mAb is able to detect human PIM2 protein by WB.

DILUTION neat supernatant

Predicted molecular weight: **34kDa, 38 kDa and 40kDa**
Observed molecular weight: **34kDa, 38 kDa and 40kDa**

LANES

Lane 1	Transfected HEK-PIM1	(10ug) (-)
Lane 2	Transfected HEK-PIM2	(10ug) (+)
Lane 3	Jurkatt	(100ug) (-)
Lane 4	Ramos	(100ug) (+)
Lane 5	RPMI-8226	(100ug) (+)
Lane 6	OPM2	(100ug) (+)
Lane 7	U266	(100ug) (+)
Lane 8	Raji	(100ug) (+)

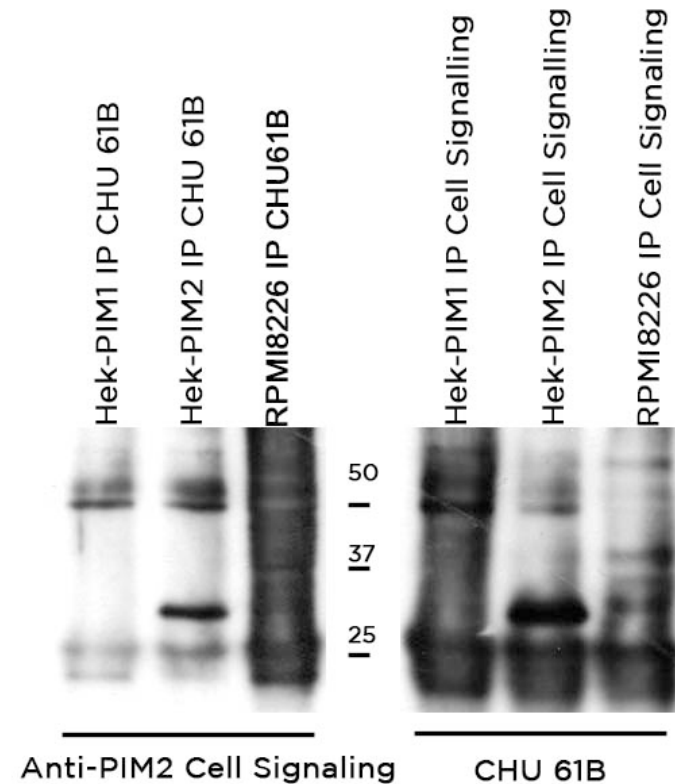


CHU61B mAb is able to detect human PIM2 protein by IP.

DILUTION neat supernatant and PIM2 (40ul/lane) and PIM2 (D1D2) rabbit mAb (Cell Signaling) was used 1:50.

RESULTS

Immunoprecipitation of protein extracts from HEK-PIM1, RPMI8226 and HEK-PIM2 with the anti-PIM2 commercial antibody (clone D1D2) and with anti-PIM2 CHU61B followed by western blotting with anti-PIM2 (D1D2) (1:500) and the CHU61B (neat). In both cases it is possible to observe a specific band of 34 kDa in HEK-PIM2 cell extract confirming antibody specificity. In case of RPMI8226 cell line immunoprecipitated using anti-PIM2 (clone D1D2) it is possible to observe the presence of three bands corresponding to the three PIM2 isoforms (34, 38, and 40 kDa).



● | IHC-P | **Immunohistochemistry (paraffin)**

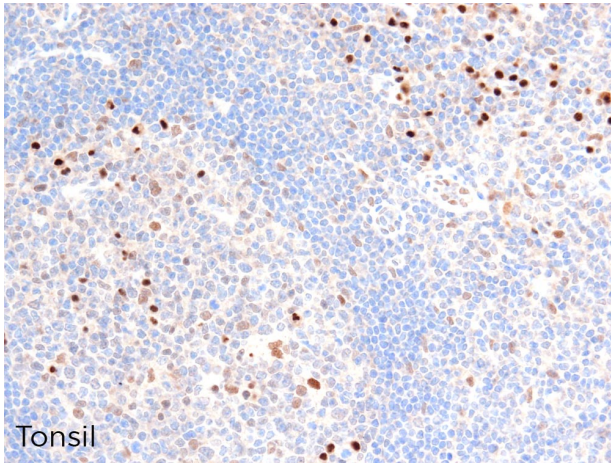
CHU61B mAb can be used to detect PIM2 protein in human paraffin tissues

TISSUE SAMPLE human tonsil

DILUTION neat supernatant

ANT. RETRIEVAL 20 minutes ER2 (Tris-EDTA)

DETECTION SYSTEM Novolink kit (BondMax Leica)



● | IF | **Immunofluorescence (paraffin) Not Recommended**

● | IHC-F | **Immunohistochemistry (frozen) Not Recommended**

● | FC | **Flow Cytometry Not tested**

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