Caspase-8 (mouse) monoclonal antibody (1G12)

This antibody is covered by our Worry-Free Guarantee.

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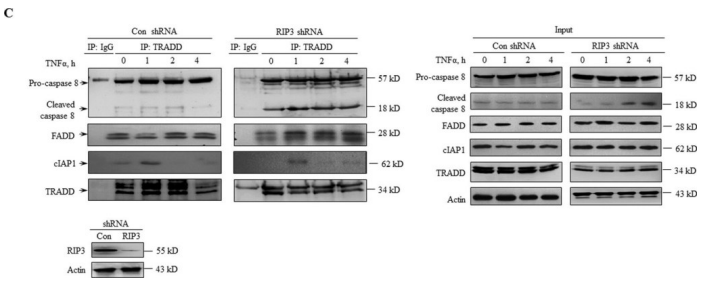
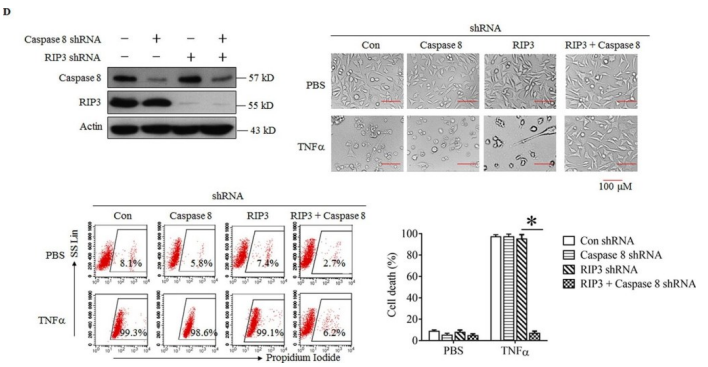
ALX-804-447-C100	100µg

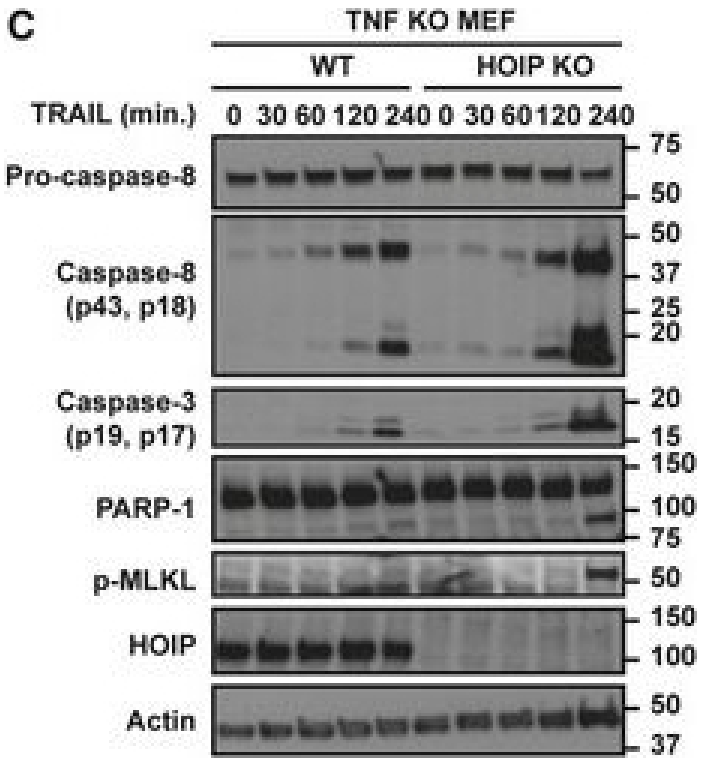
Manuals, SDS & CofA View Online »



TRADD activates the caspase pathway by binding to and activating caspase 8. (A) TRADD mediates the TNFα-induced activation of the caspase pathway in the absence of RIP3. The negative control, TRADD knockdown, RIP3 knockdown or RIP3 and TRADD double-knockdown L929 cells were treated with TNFα for the indicated times, and the cleavage of PARP and caspase 3 was assessed by western blotting. Actin was used as a loading control. The full-length blots are presented in Supplementary Figure 4A. (B) TRADD knockdown suppresses TNFα-triggered caspase 8 activation in RIP3 knockdown L929 cells. TRADD knockdown, RIP3 knockdown or RIP3 and TRADD double-knockdown L929 cells were treated with or without TNFα for 12 h and then harvested for the measurement of caspase 8 activity. *P< 0.01 compared to the control shRNA group treated with TNFα. (C) RIP3 knockdown enhances the interactions between TRADD and caspase 8. RIP3 knockdown and the negative control L929 cells were treated with TNFα for the indicated times, and the cell lysates were immunoprecipitated with a TRADD antibody. Western blotting was used to detect TRADD, caspase 8, cIAP1, FADD and Actin. The full-length blots are presented in Supplementary Figure 4C.



RIP3 knockdown switches TNFα-induced necroptosis to apoptosis in L929 cells. (A) Z-VAD blocks the TNFα-induced death of RIP3 knockdown L929 cells. The cells were infected with RIP3 shRNA or the control shRNA lentivirus, and western blotting was performed to determine the RIP3 knockdown efficiency. The full-length blots are presented in Supplementary Figure 1A. The cells were treated with TNFα or TNFα plus Z-VAD for 48 h, and cell death was measured by microscopy (200×) and flow cytometry. *P < 0.01. (B) RIP3 knockdown facilitates the TNFα-triggered activation of the caspase pathway. L929 cells were infected with the RIP3 shRNA or the negative control shRNA lentivirus and then treated with or without TNFα for an additional 12 h. Western blotting was performed to detect the knockdown efficiency and the cleavage of PARP and caspase 3. Actin was used as a loading control. The full-length blots are presented in Supplementary Figure 1B. (C) Caspase 8 activity was significantly increased in RIP3 knockdown L929 cells following TNFα stimulation. The RIP3 knockdown and negative control L929 cells were treated with or without TNFα for 12 h and then harvested to measure the activity of caspase 8. More than three independent experiments were performed for each group, and the relative activity of caspase 8 was calculated by normalizing the caspase 8 activity of all the groups with the activity of the negative control group. *P < 0.01. (D) Caspase 8 mediates the TNFα-induced death of RIP3 knockdown L929 cells. The knockdown of specific genes was mediated by infecting L929 cells with lentiviruses expressing shRNAs, and western blotting was used to evaluate the knockdown efficiency. The full-length blots are presented in Supplementary Figure 1D. The cells were treated with or without TNFα for 48 h, and cell death was measured by microscopy (200×) and flow cytometry. The RIP3 shRNA/DMSO and the RIP3 shRNA/TNFα FACS data prsented in Figure 1D are the same as that in Figure 2A. *P < 0.01.



HOIP limits TRAIL-induced apoptosis and necroptosisWT and HOIP KO TNF KO MEFs were stimulated with iz-TRAIL at the indicated concentrations for 24 h (n = 5; mean ± SEM).WT and HOIP KO TNF KO MEFs, pre-treated with zVAD and Nec-1s as indicated for 1 h, were stimulated with iz-TRAIL for 24 h (1 μg/ml) (n = 4; mean ± SEM).WT and HOIP KO TNF KO MEFs were stimulated with iz-TRAIL (1 μg/ml) for the indicated times. Lysates were analysed by Western blot.Control (CTRL) and HOIP KO K562 cells were stimulated with iz-TRAIL at the indicated concentrations for 24 h (n = 4; mean ± SEM).Control and HOIP KO K562 cells, pre-treated with zVAD as indicated for 1 h, were stimulated with iz-TRAIL (1 μg/ml) for 24 h (n = 4; mean ± SEM).Control and HOIP-deficient K562 cells were stimulated with iz-TRAIL (100 ng/ml) for the indicated times. Lysates were analysed by Western blot. Black arrowhead indicates the cleaved form of HOIP.Data information: Cell death was determined after 24 h of stimulation by flow cytometry after propidium iodide (PI) labelling. *P < 0.05, **P < 0.01, ***P < 0.001; statistics were performed using t-test. See also Appendix Fig S1.

Handling & Storage

Handling	For long term storage, aliquot and freeze at -20°C. Avoid freeze/thaw cycles.
Short Term Storage	+4°C
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	FLICE
Application	ELISA, Flow Cytometry, ICC, WB
Application Notes	ELISA: recombinant mouse caspase-8 Flow Cytometry: overexpressed casepase-8 Excellent for Western blot. Detects bands of ~55kDa (full-length caspase-8) and ~18kDa (apoptosis-induced cleavage fragment) by Western blot.
Clone	1G12
Crossreactivity	Does not cross-react with human caspase-8.
Formulation	Liquid. In PBS containing 0.02% sodium azide.
Host	Rat
Immunogen	Recombinant mouse p20 caspase-8 subunit.
Isotype	IgG1
Purity Detail	Protein G-affinity purified.
Species Reactivity	Mouse
Specificity	Recognizes the p18 subunit of caspase-8.

UniProt ID O89110

Worry-free Guarantee **This antibody is covered by our Worry-Free Guarantee .**

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