Human/Mouse/Rat HTRA2/Omi Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1458

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat full length and mitochondria-processed HTRA2/Omi.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human HTRA2/Omi Ala134-Glu458 Accession # O43464
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

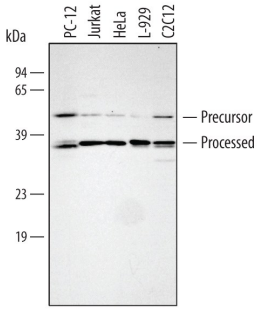
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.25 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Simple Western	2.5 µg/mL	See Below

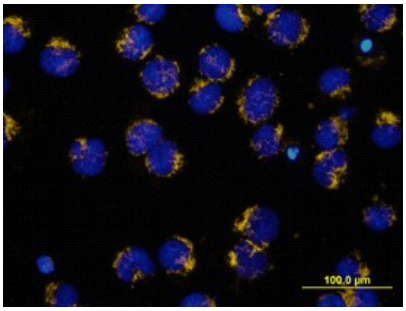
DATA

Western Blot



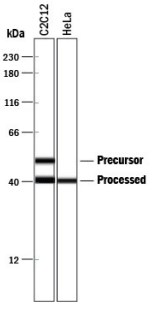
Detection of Human/Mouse/Rat HTRA2/Omi by Western Blot. Western blot shows lysates of PC-12 rat adrenal pheochromocytoma cell line, Jurkat human acute T cell leukemia cell line, HeLa human cervical epithelial carcinoma cell line, L-929 mouse fibroblast cell line, and C2C12 mouse myoblast cell line. PVDF membrane was probed with 0.25 µg/mL of Rabbit Anti-Human/Mouse/Rat HTRA2/Omi Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1458) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for HTRA2/Omi at approximately 36 and 49 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



HTRA2/Omi in Jurkat Human Cell Line. HTRA2/Omi was detected in immersion fixed Jurkat human acute T cell leukemia cell line stimulated with staurosporin using Rabbit Anti-Human/Mouse/Rat HTRA2/Omi Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1458) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (yellow; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human and Mouse HTRA2/Omi by Simple Western™. Simple Western lane view shows lysates of C2C12 mouse myoblast cell line and HeLa human cervical epithelial carcinoma cell line, loaded at 0.2 mg/mL. Specific bands were detected for HTRA2/Omi at approximately 51 kDa (precursor) and 41 kDa (processed) (as indicated) using 2.5 µg/mL of Rabbit Anti-Human/Mouse/Rat HTRA2/Omi Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1458). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

HtrA2/Omi is the mammalian homologue of bacterial high temperature requirement protein (HtrA). HtrA2/Omi localizes to the mitochondria and is processed to expose an amino-terminal Reaper-like motif similar to SMAC/Diablo. HtrA2/Omi is released from the mitochondria in response to apoptotic insult and can interact with the BIR2 or BIR3 domains of XIAP to relieve caspase-IAP inhibition. This effect can be measured by reversing XIAP-BIR2 (R&D Systems, Catalog # 786-XB) inhibition of Caspase-7 (R&D Systems, Catalog # 823-C7) cleavage of a fluorogenic peptide (DEVD-AFC, MP Bio, Catalog # AFC-138). IC_{50} values for this effect are typically between 0.2 and 1.5 µM. HtrA2/Omi is trimeric and functions as a serine protease. The serine protease activity may play a more central role in apoptosis than its IAP antagonizing function. A PDZ domain regulates the serine protease activity by blocking access to the active site. The specificity of the protease is yet to be defined and no endogenous substrates are known to date.

References:

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2. van Loo, G. *et al.* (2002) Cell Death & Diff. **9**:20.
3. Hedge, R. *et al.* (2001) J. Biol. Chem. **277**:432.
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