Mouse IL-33 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3626

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse IL-33 in ELISAs and Western blots. In sandwich immunoassays, less than 2% cross-reactivity with recombinant human IL-33 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant mouse IL-33 Ser109-Ile266 Accession # Q8BVZ5
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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 either lyophilized or as a 0.2 µm filtered solution in PBS.

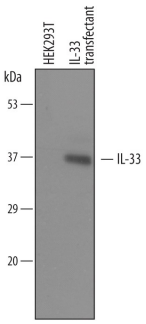
APPLICATIONS

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	0.4 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Mouse IL-33 Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Mouse IL-33 Antibody (Catalog # AF3626)
ELISA Detection	0.1-0.4 µg/mL	Mouse IL-33 Biotinylated Antibody (Catalog # BAF3626)
Standard		Recombinant Mouse IL-33 (Catalog # 3626-ML)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize IL-33-induced proliferation in the D10.G4.1 mouse helper T cell line. Schmitz, J. *et al.* (2005) Immunity **23**:479. The Neutralization Dose (ND_{50}) is typically 10-50 ng/mL in the presence of 0.25 ng/mL Recombinant Mouse IL-33.	

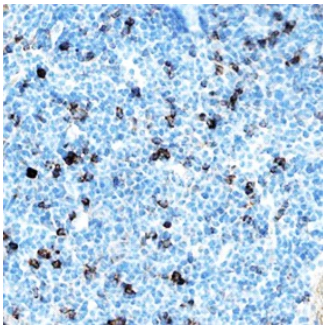
DATA

Western Blot



Detection of Mouse IL-33 by Western Blot. Western blot shows lysates of HEK293T human embryonic kidney cell line either mock transfected or transfected with full length mouse IL-33. PVDF membrane was probed with 0.4 µg/mL of Goat Anti-Mouse IL-33 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3626) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for IL-33 at approximately 37 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



IL-33 in Mouse Spleen. IL-33 was detected in immersion fixed frozen sections of mouse spleen using Goat Anti-Mouse IL-33 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3626) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in lymphocytes. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

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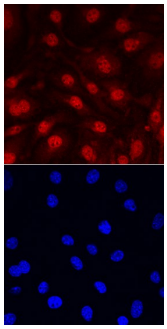
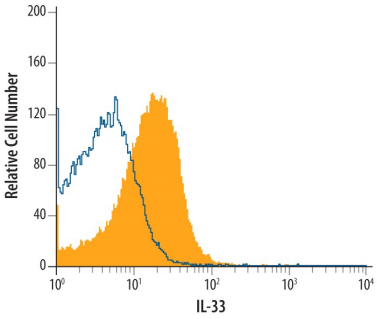
Immunocytochemistry



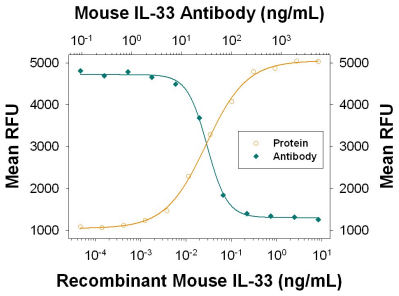
IL-33 in bEnd.3 Mouse Cell Line. IL-33 was detected in immersion fixed bEnd.3 mouse endothelioma cell line using Goat Anti-Mouse IL-33 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3626) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red, upper panel; Catalog # NL001) and counterstained with DAPI (blue, lower panel). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of IL-33 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes were treated for 5 hours with 50 ng/mL PMA and 1 µg/mL Ca^{2+} ionomycin then stained with Goat Anti-Mouse IL-33 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3626, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Neutralization



Proliferation Induced by IL-33 and Neutralization by Human IL-33 Antibody. Recombinant Mouse IL-33 (Catalog # 3626-ML) stimulates proliferation in the D10.G4.1 mouse helper T cell line in a dose-dependent manner (orange line), as measured by Resazurin (Catalog # AR002). Proliferation elicited by Recombinant Mouse IL-33 (0.25 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse IL-33 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3626). The ND_{50} is typically 10-50 ng/mL.

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

IL-33, also known as NF-HEV and DVS 27, is a 30 kDa proinflammatory protein that may also regulate gene transcription (1-3). DVS 27 was identifed as a gene that is upregulated in vasospastic cerebral arteries (1). NF-HEV was described as a nuclear factor that is preferentially expressed in the endothelial cells of high endothelial venules relative to endothelial cells from other tissues (2). IL-33 was identified based on sequence and structural homology with IL-1 family cytokines (3). DVS 27, NF-HEV, and IL-33 share 100% amino acid sequence identity. IL-33 is constitutively expressed in smooth muscle and airway epithelia. It is upregulated in arterial smooth muscle, dermal fibroblasts, and keratinocytes following IL-1α or IL-1β stimulation (1, 3). Similar to IL-1, IL-33 can be cleaved *in vitro* by caspase-1, generating an N-terminal fragment that is slightly shorter than the C-terminal fragment (3, 4). The N-terminal portion of full length IL-33 contains a predicted bipartite nuclear localization sequence and a homeodomain-like helix-turn-helix DNA binding domain. By immunofluorescence, full length IL-33 localizes to the nucleus in HUVECs and transfectants (2). The C-terminal fragment, corresponding to mature IL-33, binds and triggers signaling through mast cell IL-1 R4/ST2L, a longtime orphan receptor involved in the augmentation of Th2 cell responses (3, 5-7). A ternary signaling complex is formed by the subsequent association of IL-33 and ST2L with IL-1R AcP (8). Stimulation of Th2 polarized lymphocytes with mature IL-33 *in vitro* induces IL-5 and IL-13 secretion (3). *In vivo* administration of mature IL-33 promotes increased production of IL-5, IL-13, IgE, and IgA, as well as splenomegaly and inflammatory infiltration of mucosal tissues (3). Full length and mature mouse IL-33 share approximately 55% and 90% aa sequence identity with human and rat IL-33, respectively. Mouse IL-33 shares less than 25% aa sequence identity with other IL-1 family proteins.

References:

1. Onda, H. *et al.* (1999) J. Cereb. Blood Flow Metab. **19**:1279.
2. Baekkevold, E.S. *et al.* (2003) Am. J. Pathol. **163**:69.
3. Schmitz, J. *et al.* (2005) Immunity **23**:479.
4. Black, R.A. *et al.* (1989) J. Biol. Chem. **264**:5323.
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