

## Product datasheet for **AM26652AF-N**

### IL18 (37-194) Mouse Monoclonal Antibody [Clone ID: 91D8]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	91D8
Applications:	IP
Recommended Dilution:	Immunoprecipitation: 1 µg/sample. For details see protocol below. Not recommended for Western blot.
Reactivity:	Rat
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Mature rat IL-18 fusion protein corresponding to 37-194 aa
Specificity:	This antibody reacts with rat IL-18.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	interleukin 18
Database Link:	<a href="#">Entrez Gene 29197 Rat P97636</a>



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**Background:**

Interleukin 18 (IL-18) is an 18-kDa cytokine which identified as a costimulatory factor for production of interferon- $\gamma$  (IFN- $\gamma$ ) in response to toxic shock and shares functional similarities with IL-12. IL-18 is synthesized as a precursor 24-kDa molecule without a signal peptide and must be cleaved to produce an active molecule. IL-1 converting enzyme (ICE, Caspase-1) cleaves pro-IL-18 at aspartic acid in the P1 position, producing the mature, bioactive peptide that is readily released from the cells. It is reported that IL-18 is produced from Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts, adrenal cortex cells and murine diencephalon. IFN- $\gamma$  is produced by activated T or NK cells and plays critical roles in the defense against microbial pathogens. IFN- $\gamma$  activates macrophages and enhances NK activity and B cell maturation, proliferation and Ig secretion. IFN- $\gamma$  also induces expression of MHC class I and II antigens and inhibits osteoclast activation. IL-18 acts on T helper type-1 (Th1) T cells and in combination with IL-12 strongly induces them to produce IFN- $\gamma$ . Pleiotropic effects of IL-18 have also been reported, such as, enhancement production of IFN- $\gamma$  and GM-CSF in peripheral blood mononuclear cells, production of Th1 cytokines, IL-2, GM-CSF and IFN- $\gamma$  in T cells, enhancement of Fas ligand expression by Th1 cells.

**Synonyms:**

IL-18, IGIF, IL1F4, Iboctadekin, IL1 gamma, IL-1 gamma

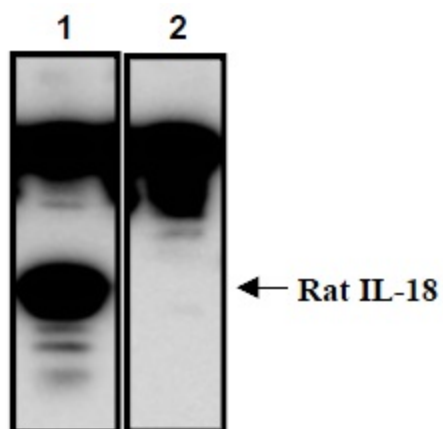
**Note:** This product was originally produced by MBL International.

**Protocol:**

**Immunoprecipitation**

- 1) Add primary antibody as suggest in the APPLICATIONS into the recombinant protein. Mix well and incubate with gentle agitation for 30-120 minutes at 4oC.
- 2) Add 10  $\mu$ L of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4oC.
- 3) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 4) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 5) Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
- 7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
- 8) Incubate the membrane with 1  $\mu$ g/mL of anti-rat IL-18 monoclonal antibody as primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 10) Incubate the membrane with the 1:1,000 mouse True Blot diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 11) Wash the membrane with PBS-T (5 minutes x 6 times).
- 12) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 13) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 14) Expose to an X-ray film in a dark room for 2 minutes.
- 15) Develop the film as usual. The condition for exposure and development may vary.

## Product images:



Immunoprecipitation of recombinant mature rat IL-18 with AM26652AF-N (1) or normal mouse IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-rat IL-18 monoclonal antibody.