

therapeutic or diagnostic purposes in humans or animals.

**Source:** Recombinant mouse  $_{\text{Hiss}}$ L-27 (m $_{\text{Hiss}}$ L-27) is a heterodimer that is composed of EBI3 Tyr19-Pro228 (Accession# NP\_056581) linked to p28 Phe29-Ser234 (Accession# NP\_663611) via the linker GGGSGGGSGGGSGGGSGGGS. m $_{\text{Hiss}}$ L-27 was expressed in human 293 cells at Cell Signaling Technology.

**Molecular Characterization:** Recombinant N-terminally His6-tagged mIL-27 has a calculated MW of 50,551. DTT-reduced and non-reduced protein migrate as 55 kDa polypeptides. The expected amino terminus of recombinant  $m_{\text{Hisf}}$ IL-27 was verified by amino acid sequencing.

**Endotoxin:** Less than 0.01 ng endotoxin/1 $\mu$ g m<sub>His6</sub>IL-27.

**Purity:** >97% as determined by SDS-PAGE of 6  $\mu$ g reduced (+) and non-reduced (-) recombinant m<sub>His6</sub>L-27. All lots are greater than 97% pure.



The purity of recombinant  $m_{\rm High}L$ -27 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant  $m_{\rm High}L$ -27 and staining overnight with Coomassie Blue.

**Bioactivity:** The bioactivity of  $m_{His6}$ IL-27 was determined in a virus protection assay. The ED<sub>50</sub> of each lot is between 1-6 ng/ml.

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Western blot analysis of extracts from Hep G2 cells, untreated or treated with m<sub>Hist</sub>L-27 for 15 min, using Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (upper) or Stat3 Antibody #9132 (lower).



The bioactivity of recombinant  $m_{\rm HSS}$ /L-27 was determined in a virus protection assay. Hep G2 cells were pre-treated with increasing concentrations of  $m_{\rm HSS}$ /L-27 for 24 hr. Cells were then innoculated with encephalomyocarditis virus (EMCV) and incubated for an additional 24 hr. Surviving cells were fixed and stained with crystal violet and the OD<sub>xos</sub> was determined.

The bioactivity of recombinant m<sub>Hist</sub>L-27 was determined by induction of MxA mRNA. Hep G2 cells were pre-treated with increasing concentrations of m<sub>Hist</sub>L-27 for 5 hr. Induction of MxA gene expression was determined by qPCR. Formulation: With carrier: Lyophilized from a 0.22  $\mu m$  filtered solution of PBS, pH 7.2 containing 20  $\mu g$  BSA per 1  $\mu g$  m\_{Hisb}IL-27.

Carrier free: Lyophilized from a 0.22  $\mu m$  filtered solution of PBS, pH 7.2.

## **Reconstitution:**

With carrier: Add sterile PBS, or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final  $m_{\rm High}$ L-27 concentration of greater than 50  $\mu$ g/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS, or PBS containing protein to minimize absorption of  $m_{\text{Hisb}}$ L-27 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock  $m_{\text{Hisb}}$ L-27 should be greater than 50 µg/ml.

**Storage:** Stable in lyophilized state at 4°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

**Applications:** Optimal concentration for the desired application should be determined by the user.

**Background:** IL-27 is a heterodimer that consists of p28 and EBI3 (1). Antigen presenting cells including monocytes, macrophages, and dendritic cells are the primary sources of IL-27 (1). IL-27 is a pleiotropic cytokine with both pro- and anti-inflammatory activities. IL-27 suppresses TH17 responses and promotes type I regulatory cell differentiation (2-4). In contrast, IL-27 has been shown to play a pro-inflammatory role in a mouse model of T cell-induced colitis (5). IL-27 may also have anti-viral activities (6). IL-27 exerts its effects through a heterodimeric receptor consisting of WSX-1 and gp130 (1,7). IL-27-induced signaling results in Stat1 and Stat3 phosphorylation (1,7).

## **Background References:**

- (1) Pflanz, S. et al. (2002) Immunity 16, 779-90.
- (2) Villarino, A.V. et al. (2010) J Immunol 185, 6461-71.
- (3) Apetoh, L. et al. (2010) Nat Immunol 11, 854-61.
- (4) Murugaiyan, G. et al. (2010) Proc Natl Acad Sci U S A 107, 11495-500.
- (5) Cox, J.H. et al. (2011) J Exp Med 208, 115-23.
- (6) Bender, H. et al. (2009) Hepatology 50, 585-91.
- (7) Pflanz, S. et al. (2004) J Immunol 172, 2225-31.