

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human IL-35 protein			
	Human IL-27β/EBI3 (Arg21-Lys229) Accession # Q14213	(GGGS) ₄	Human IL-12α/p35 Arg23-Ser219 Accession # P29459	IEGRMD
				Human IgG ₁ Pro100-Lys330

N-terminal Sequence Arg21

Analysis

Structure / Form Disulfide-linked homodimer

Predicted Molecular Mass 73 kDa

Mass

SPECIFICATIONS

SDS-PAGE	81-97 kDa, reducing conditions
Activity	Measured by its binding ability in a functional ELISA. When Recombinant Human IL-12 Rβ2 Fc Chimera (Catalog # 1959-B2) is immobilized at 5 μg/mL (100 μL/well), the concentration of Recombinant Human IL-35 Fc Chimera that produces 50% optimal binding response is approximately 20-120 ng/mL.
Endotoxin Level	<0.30 EU per 1 μg of the protein by the LAL method.
Purity	>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 μg/mL in PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 3 months, -20 to -70 °C under sterile conditions after reconstitution.

DATA

<p>Bioactivity</p> <p>When Recombinant Human IL-12 R beta 2 Fc Chimera (Catalog # 1959-B2) is immobilized at 5 μg/mL (100 μL/well), Recombinant Human IL-35 Fc Chimera (Catalog # 8608-IL) binds with an ED₅₀ of 20-120 ng/mL. Recombinant Human IL-35 from the two competitors have much weaker Recombinant Human IL-12 R beta 2 Fc Chimera binding activity.</p>	<p>SDS-PAGE</p> <p>1 μg/lane of Recombinant Human IL-35 Fc Chimera Protein from R&D Systems and two competitors was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining. Recombinant Human IL-35 Fc Chimera Protein (Catalog # 8608-IL) from R&D Systems shows single bands at 85 kDa and 185 kDa, respectively. The R&D Systems Protein offers a better purity than the competition.</p>
--	---

BACKGROUND

Interleukin 35 (IL-35) is a member of the IL-12 family of heterodimeric cytokines. Unlike other IL-12 family cytokines which stimulate the immune response, the predominant function of IL-35 is as an immunosuppressant. IL-12 cytokines are composed of an α and β subunit which, for IL-35 are the IL-12 p35 subunit and the EB13 subunit, respectively (1-3). The IL-12 p35 subunit of IL-35 is synthesized as a 219 amino acid (aa) precursor protein with a 22 aa signal sequence and a 197 aa mature region. The EB13 subunit of IL-35 is synthesized as a 229 aa precursor protein that contains a 20 aa signal sequence and a 209 aa mature region. Human and mouse IL-35 share 58% and 62% sequence homology in their IL-12 p35 and EB13 subunits, respectively. IL-35 binds to the homodimeric receptors, IL-12 R β 2 and gp130, as well as to the IL-12 R β 2-gp130 receptor heterodimer (4). The expression pattern of IL-35 is thought to differ between mouse and humans (5). In mouse regulatory T cells, both subunits of IL-35 are constitutively expressed and the mature IL-35 is secreted. In humans, IL-12 p35 is the only subunit constitutively expressed in regulatory T cells. Immune activation can induce EB13 expression and IL-35 secretion in human effector T cells (6-8). IL-35 is also expressed and secreted in human placental trophoblasts (1, 9). In both human and mouse IL-35 has been shown to suppress effector T cell proliferation, inhibit Th17 cell development, and promote the conversion of T cells and B cells into regulatory T and B cells, respectively (1, 4, 8, 10, 11). IL-35 is thought to be involved in infectious tolerance and inflammatory cytokine-mediated autoimmune disorders (1, 3, 5, 12). Serum levels of IL-35 are associated with acute graft-versus-host disease following hematopoietic stem cell transplantation (13, 14). IL-35 also functions as a regulator of tumor growth (2, 12, 15).

References:

1. Collison, L.W. and D.A. Vignali (2008) *Immunol. Rev.* **226**:248.
2. Wang, Z. *et al.* (2013) *J. Immunol.* **190**:2415.
3. Choi, J. *et al.* (2015) *Clin. Rev. Allergy. Immunol.*
4. Collison, L.W. *et al.* (2012) *Nat. Immunol.* **13**:290.
5. Ning-Wei, Z. (2010) *Rev. Med. Chil.* **138**:758.
6. Bardel, E. *et al.* (2008) *J. Immunol.* **181**:6898.
7. Guttek, K. and D. Reinhold (2013) *Cytokine* **64**:46.
8. Collison, L.W. *et al.* (2007) *Nature* **450**:566.
9. Mao, H. *et al.* (2013) *Hum. Immunol.* **74**:872.
10. Wang, R.X. *et al.* (2014) *Nat. Med.* **20**:633.
11. Niedbala, W. *et al.* (2007) *Eur. J. Immunol.* **37**:3021.
12. Collison, L.W. *et al.* (2010) *Nat. Immunol.* **11**:1093.
13. Liu, Y. *et al.* (2014) *Leukemia*. [Epub ahead of print; PMID: 25363669].
14. Zhang, X. *et al.* (2014) *Ann. Hematol.* [Epub ahead of print; PMID: 25512184].
15. Long, J. *et al.* (2013) *Biochem. Biophys. Res. Commun.* **430**:364.