

## Product Information

### ANTI-gp130 Developed in Rabbit, IgG Fraction of Antiserum

Product Number **G4165**

#### Product Description

Anti-gp130 is developed in rabbit using a recombinant GST-gp130 containing the cytoplasmic domain (amino acids 713-918) of human gp130 as immunogen. The IgG fraction of antiserum is purified using protein A.

Anti-gp130 specifically recognizes gp130 in both human and mouse by immunoblotting and immunoprecipitation.

Interleukin-6 exerts its activity through binding to a high affinity receptor complex consisting of two membrane glycoproteins: an 80 kD IL-6-binding receptor protein (IL-6R) and a 130kD signal-transducing protein consisting of a 19 amino acid residue signal peptide (gp130). IL-6 is a highly pleiotropic cytokine which plays a critical role in the immune response, in acute phase reactions and in hemopoiesis.<sup>1-3</sup> It is produced by T and B cells, macrophages, bone marrow stromal cells, fibroblasts, and endothelial cells.<sup>1</sup> IL-6 exerts a multitude of biological activities on a wide variety of cell types. These include the induction of B cell differentiation for Ig production, the stimulation of plasmacytoma and B cell hybridoma growth, the stimulation of thymocyte and T-cell proliferation and the induction of T cell differentiation to cytotoxic T lymphocytes. A naturally occurring soluble form of the IL-6R, which binds IL-6 and which mediates IL-6 signaling through interaction with gp 130, is identified in the urine of adult humans as well as human and mouse serum.

#### Reagents

Anti-gp130 is supplied as the IgG fraction of antiserum 0.07 M Tris-glycine, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.035% sodium azide. Antibody concentration is approximately 0.7 mg/ml.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

#### Storage/Stability

Store at 0°C to -20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

#### Procedure

##### *Immunoprecipitation*

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1mg/ml total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 : g of Anti-gp130 (G 4165) to 500 : g – 1 mg cell lysate.
3. Gently rock the reaction mixture at 4EC overnight.
4. Capture the immunocomplex by adding 100 : l of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 : l packed beads) (Sigma Product No. P2545).
5. Gently rock reaction mixture at 4EC for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 : l 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

##### *Lysis Buffer:*

50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na<sub>3</sub>VO<sub>4</sub>, and 1mM NaF.

#### Product Profile

Recommended working concentration is 0.5-2µg/ml by immunoblotting using RIPA lysates from mouse 3T3 fibroblasts, anti-rabbit IgG-peroxidase conjugate and a chemiluminescent detection system.

Recommended working concentration for IP is 4 µg to immunoprecipitate gp130 from 500 µg of mouse 3T3 fibroblast RIPA lysate.

#### References

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3. Van Snick, J., Ann Rev. Immunol., **8**, 253,(1990).
4. Ward, L.d., et al., J. Biol. Chem., **269**, 23286 (1994).
5. Klein, B., et al., Leuk. Lymphoma, **34**, 63 (1999).

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