Product Datasheet

IL-6 Antibody
NB600-1131

Unit Size: 0.2 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Updated 8/21/2017 v.20.1

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## NB600-1131
### IL-6 Antibody

#### Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit Size</strong></td>
<td>0.2 ml</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>This product is unpurified. The exact concentration of antibody is not quantifiable.</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>No Preservative</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Unpurified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>24 kDa</td>
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#### Product Description

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<tr>
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<tbody>
<tr>
<td><strong>Host</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Gene ID</strong></td>
<td>3569</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>IL6</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td><strong>Reactivity Notes</strong></td>
<td>Cross-reactivity reported in scientific literature: Mouse - PMID 23015436; Rat - PMID 25058444</td>
</tr>
<tr>
<td><strong>Specificity/Sensitivity</strong></td>
<td>This antiserum detects recombinant and native IL-6 present in body fluids and cell supernatants in various assays (ie. IL-1 stimulated IL-6 production from fibroblasts). In Western blot analysis of natural cell products or human body fluids, multiple bands of IL-6 will appear due to the variable amount of glycosylation on the molecule. The antiserum is also useful for neutralization of human of IL-6 activity in bioassays. For neutralization, incubate the sample with a 1:400 dilution of the antiserum for at least 4 hours before being tested. A control of similarly diluted normal rabbit IgG (heat inactivated) is recommended. In neutralization experiments in vitro, this antibody does not result in enhanced activity of IL-6. However, because antibodies to IL-6 may act as a soluble receptor in vivo, some antibodies to IL-6 act as carriers and enhance IL-6 activity.</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Recombinant human IL-6 produced in E.coli.</td>
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#### Product Application Details

<table>
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</thead>
<tbody>
<tr>
<td><strong>Applications</strong></td>
<td>Western Blot, ELISA, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
<tr>
<td><strong>Recommended Dilutions</strong></td>
<td>Western Blot 1:500 - 1:2000, ELISA 1:1000 - 1:5000, Immunohistochemistry 1:400 - 1:800, Immunocytochemistry/Immunofluorescence 1:10 - 1:500, Immunoprecipitation 1:400 - 1:800, Immunohistochemistry-Paraffin 1:400 - 1:800</td>
</tr>
</tbody>
</table>
This antiserum against IL-6 has been tested for use in neutralizations, ELISA, immuno-histochemistry, radioimmunoassay, immunoprecipitation and immunoblotting. Reactivity in other immunoassays is unknown. For immunoblotting use the supernatants or lysates of 2 x 10^6 endotoxin-stimulated human peripheral blood mononuclear cells. PBMC are stimulated for 24 hours with 1% human serum plus 10 ng/mL E.coli LPS. For immunohistochemistry either paraffin fix or cryofix tissue. For immunoprecipitation, pre-clearing with a non-specific rabbit IgG is helpful - reduce background. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Western Blot: IL-6 Antibody [NB600-1131] - Protein was resolved on a 4-20% Tris-Glycine gel by SDS-PAGE and transferred onto nitrocellulose. The blot shows detection of a band 21 kDa in size corresponding to anti-IL6 antibody. Molecular weight markers are also shown (MW). After transfer, the membrane was blocked for 30 minutes with 1% BSA-TBST. Detection occurred using peroxidase conjugated anti-Rabbit IgG secondary antibody diluted 1:40,000 in blocking buffer for 30 min at RT followed by reaction with FemtoMax chemiluminescent substrate.

Immunocytochemistry/Immunofluorescence: IL-6 Antibody [NB600-1131] - IL-6 antibody was tested in Raw264.7 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).

Immunohistochemistry-Paraffin: IL-6 Antibody [NB600-1131] - IHC staining of IL6 in human bladder cancer using DAB with hematoxylin counterstain.
Western Blot: IL-6 Antibody [NB600-1131] - Immunoblot using anti-IL6 antibody. This blot shows detection of an IL-6-GST fusion protein (300 ng, lane 1, green, 800 nm channel). After blocking the membrane was probed with the primary antibody diluted to 1:1,000. Incubation was overnight at 4 degrees C followed by washes and reaction with a 1:10,000 dilution of IRDye 800 conjugated Gt-a-Rabbit IgG [H&L] MX10 for 45 min at room temperature. Molecular weight markers are shown for size comparison (lane M, red, 700 nm channel). IRDye 800 fluorescence image was captured using the Odyssey Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

Western Blot: IL-6 Antibody [NB600-1131] - Analysis using the HRP conjugate of NB600-1131. Detection of Lane 1: Human IL-6. Detection of Lane 2: none. Load: 50 ug per lane. Primary antibody: none. Secondary antibody: Peroxidase Human IL-6 secondary antibody at 1:1,000 for 60 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 20 kDa for Human IL-6.
### Publications


Details:

TLR4 Inhibitor Peptide Set was used for functional assays in experiments involving human first-trimester trophoblast cells (Sw.71), isolated from a normal 7-week placenta. The cells were stimulated with either advanced glycation end products/AGEs or high-mobility group box-1/HMGB1 in the absence or presence of either RAGE antagonist FPS-XM1 or TLR4 inhibitor peptides.


Details:

IL6 antibody used in IHC-P for detecting the inflammation of the gingival connective tissue of Rats inoculated or not with Porphyromonas gingivalis GroEL (inoculated into rat gingiva) - Figure 5B.


Procedures

Immunohistochemistry-Paraffin Protocol Specific for NB600-1131: IL6 Antibody (NB600-1131)

Materials

1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 4.3mmol/L, KH2PO4 1.4 mmol/L
2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
3) 3% Hydrogen peroxide
4) Primary antibody
5) Blocking serum (normal serum)
6) Biotinylated secondary antibody
7) DAB staining kit

Methods

1. Dewax and hydration of slides using xylene and EtOH:
   Dry slides for 20 min in a 60°C oven
   Add Xylene, 2 x 10 min
   100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration
   Rinse in PBS, 5'

2 Antigen retrieval method (only for paraffin slides)
1a. High-pressure antigen retrieval procedure (recommended method)
   Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0) within a pressure cooker
   Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the required high-pressure will have been reached, and slides will be incubated for 2 min.
   Turn off heat, and allow buffer and slides to cool to room temperature
   Slides are then rinsed in PBS for 5 minutes
2. Add 3% hydrogen peroxide solution, 10’at RT, then PBS, 3X5'
3. Normal blocking serum, 20’at RT
4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C
5. Rinse with PBS, 3 X 5’ each rinse
6. Add Biotin-conjugated second antibody, 10’at RT
7. Rinse with PBS, 3 X 5’ each rinse
8. Add Streptavidin-Peroxidase, 10’at RT
9. Rinse with PBS, 3 X 5’ each rinse
10. Staining with DAB solution, 2-5’under microscope
11. Stop the reaction by washing in tap water
12. Counterstain in Haematoxylin for 3-5 minutes
13. 75%, 80%, 95% and 100% ethanol, 5x2’, xylene 2 x 10’
Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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