MOUSE ANTI RAT CD4 (IgG)  

Clone Number: W3/25
Volume/Quantity: 1 mg
CD Number: CD4
Product Form: Purified IgG - liquid
Preparation: Purified IgG prepared by affinity chromatography on Protein A from tissue culture supernatant
Buffer: Phosphate buffered saline pH7.4
Preservatives Stabilisers: 0.1% Sodium Azide (NaN₃)

Approx. Protein Concentrations: IgG concentration 1 mg/ml

Immunogen: Rat Thymocyte Membrane Preparation

Fusion Partners: Spleen cells from immunised BALB/c mice were fused with cells of the NS-1 mouse myeloma cell line.

Isotype: IgG1 (Mouse)

Specificity: MCA55 recognises the rat CD4 cell surface glycoprotein, a 55kD molecule expressed by helper T cells, thymocytes and macrophages. This antibody inhibits proliferation and IL-2 production in the MLR reaction.

*This clone has been described reacting with paraffin-embedded material following PLP fixation (periodate-lysine-paraformaldehyde) - see Whiteland et al. (1995).

Applications:  

Yes  No  Not Tested  Suggested Working Dilution  
Flow Cytometry  
Immunohistology - frozen  
- paraffin  
- resin  
ELISA  
Immunoprecipitation  
Western Blotting  
Radioimmunoassay  

Where this antibody has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the antibody for use in their own system using appropriate negative/positive controls.

Flow Cytometry: Use 10μl of the suggested working dilution to label 10⁶ cells.

Method sheets are available on request.
Recommended Secondary Reagents:
- Goat anti mouse IgG FITC conjugate (non-cross reacting with rat IgG) - STAR70
- Goat anti mouse IgG RPE conjugate (non-cross reacting with rat IgG) - STAR76

Recommended Negative Controls:
Mouse IgG1 Negative Control - MCA1209

References:

Storage Conditions: Store at +4°C for one month or at -20°C for longer.

Avoid repeated freezing and thawing as this may denature the antibody.

Should this product contain a precipitate we recommend microcentrifugation before use.

Shelf Life: 12 months from date of despatch.

Health and Safety Information: (A full Health and Safety assessment is available upon request)

This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
caspase-1 p20 (M-19): cat # sc-1218

BACKGROUND

Caspase-1 (also known as Interleukin-1β converting enzyme or ICE) is primarily expressed in monocytes and functions as a cysteine protease to process the 33 kDa inactive precursor of interleukin-1β to the 17 kDa pro-inflammatory cytokine, IL-1β (1-3). Considerable interest in caspase-1 is due to its implication in regulated programmed cell death (apoptosis) in neuronal cells (4,5). For instance, caspase shares 28% sequence homology with Ced-3, a C. elegans protein in the apoptosis pathway, and overexpression of caspase-1 in rat fibroblasts causes cell death (4,5). In its active form, caspase-1 is an oligomeric enzyme with 20 kDa (p20) and 10 kDa (p10) subunits derived from a 45 kDa pro-enzyme (p45) by cleavage at four Asp-Xaa bonds, Asp 103-Ser 104, Asp 119-Asn 120, Asp 297-Ser 298 and Asp 316-Ala 317 (6).

SOURCE
caspase-1 p20 (M-19) is provided as either a goat (sc-1218) or rabbit (sc-1218-R) affinity-purified polyclonal antibody raised against a peptide mapping at the carboxy terminus of the p20 subunit of caspase-1 (IL-1β converting enzyme) of mouse origin.

PRODUCT

Each vial contains 200 µg goat IgG (sc-1218) or 200 µg rabbit IgG (sc-1218-R) in 1.0 ml of PBS containing 0.1% sodium azide and 0.2% gelatin. Blocking peptide is available for competition studies (cat # sc-1218 P) (100 µg peptide in 0.5 ml PBS with 0.1% sodium azide and 100 µg BSA).

SPECIFICITY
caspase-1 p20 (M-19) reacts with the p20 subunit and precursor of caspase-1 (also designated ICE, for IL-1β converting enzyme) of mouse and rat origin by Western blotting and immunohistochemistry; non cross-reactive with caspase p10.

Recommended dilution for Western blot analysis: 1:100-1:1000 (goat polyclonal); 1:200-1:1000 (rabbit polyclonal).

STORAGE

Store at 4°C, do not freeze; stable for one year from the date of shipment.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

REFERENCES


### Western Blotting Secondary Antibodies and Luminol Reagent

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Conjugate</th>
<th>Amount</th>
<th>Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-rabbit IgG-HRP</td>
<td>sc-2004</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-mouse IgG-HRP</td>
<td>sc-2005</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-rat IgG-HRP</td>
<td>sc-2006</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
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<tr>
<td>anti-goat IgG-HRP</td>
<td>sc-2020</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-rabbit IgG-AP</td>
<td>sc-2007</td>
<td>alkaline phosphatase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-mouse IgG-AP</td>
<td>sc-2008</td>
<td>alkaline phosphatase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
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<tr>
<td>anti-rat IgG-AP</td>
<td>sc-2021</td>
<td>alkaline phosphatase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-goat IgG AP</td>
<td>sc-2022</td>
<td>alkaline phosphatase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
</tbody>
</table>

**Luminol Reagent**

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminol Reagent</td>
<td>sc-2048</td>
<td>sufficient for 2,000 cm² membrane area</td>
</tr>
</tbody>
</table>

The above secondary reagents are suitable for chemiluminescence Western blotting applications. Not for use with Cruz Marker molecular weight markers. Santa Cruz Biotechnology's Western Blotting Chemiluminescence “Luminol” Reagent is recommended for Western blotting enhanced chemiluminescence applications using Horseradish Peroxidase (HRP) conjugated secondary antibodies.

### Cruz Marker™ Reagents

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Conjugate</th>
<th>Amount</th>
<th>Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-rabbit IgG-HRP</td>
<td>sc-2030</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-mouse IgG-HRP</td>
<td>sc-2031</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-rat IgG-HRP</td>
<td>sc-2032</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
<tr>
<td>anti-goat IgG-HRP</td>
<td>sc-2033</td>
<td>peroxidase</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:500-1:2000</td>
</tr>
</tbody>
</table>

**Cruz Marker MW Standards**

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Molecular Weight Standards</th>
<th>Number of Gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cruz Marker MW Standards</td>
<td>sc-2035</td>
<td>132 kDa, 90 kDa, 55 kDa, 48 kDa, 34 kDa, 23 kDa</td>
<td>50</td>
</tr>
</tbody>
</table>

The above secondary reagents include antibody directed to an epitope common to each of the Cruz Marker molecular weight standards and are thus suitable for chemiluminescence Western blotting applications using Cruz Marker standards for molecular weight determination. Alkaline phosphatase (AP) conjugated Cruz Marker compatible secondary antibodies are also available. Cruz Markers are provided for use as internal molecular weight standards for chemiluminescence Western blotting applications and must be used in combination with Cruz Markers compatible Western blotting secondary antibodies available only from Santa Cruz Biotechnology, Inc.

### Fluorescein- and Biotin-Conjugated Secondary Antibodies

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Conjugate</th>
<th>Amount</th>
<th>Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-rabbit IgG-FITC</td>
<td>sc-2012</td>
<td>fluorescein</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-mouse IgG-FITC</td>
<td>sc-2010</td>
<td>fluorescein</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-rat IgG-FITC</td>
<td>sc-2011</td>
<td>fluorescein</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-goat IgG-FITC</td>
<td>sc-2024</td>
<td>fluorescein</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-rabbit IgG-B</td>
<td>sc-2040</td>
<td>biotin</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-mouse IgG-B</td>
<td>sc-2039</td>
<td>biotin</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-rat IgG-B</td>
<td>sc-2041</td>
<td>biotin</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
<tr>
<td>anti-goat IgG-B</td>
<td>sc-2042</td>
<td>biotin</td>
<td>200 µg</td>
<td>0.5 ml</td>
<td>1:100-1:400</td>
</tr>
</tbody>
</table>

Fluorescein-conjugated and biotin-conjugated secondary antibodies are optimized for use in immunohistochemical staining procedures. The anti-rabbit IgG, anti-rat IgG, and anti-goat IgG antibodies are pre-absorbed with human and mouse IgG; the anti-mouse IgG antibodies are pre-absorbed with human IgG. Anti-rabbit, mouse and rat IgGs are raised in goat; anti-goat IgGs are raised in donkey.
Monoclonal Anti-human IL-6 Antibody

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, E. coli-derived, recombinant human interleukin 6 (rIL-6). The IgG fraction of ascites fluid was purified by Protein A chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 μg/mL.

Storage
Lyophilized samples are stable for twelve months from date of receipt when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. Avoid repeated freeze-thaw cycles.

Specificity
This antibody has been selected for its ability to neutralize human IL-6 bioactivity and for use as a capture antibody in sandwich ELISAs.

Applications
Neutralization of Human IL-6 Bioactivity - The exact concentration of antibody required to neutralize human IL-6 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The Neutralization Dose50 (ND50) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response. As shown in figures 1 and 2, the ND50 for this lot of anti-human IL-6 antibody was determined to be approximately 0.05 - 0.15 μg/mL in the presence of 25 ng/mL of rIL-6, using proliferation of the IL-6-dependent murine plasmacytoma cell line, T1165.85.2.1 as an assay. The specific conditions are described in the figure legends.

Western Blot - This antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect human IL-6. The detection limit for rIL-6 is approximately 2 ng/facne under non-reducing and reducing conditions. In western blots, this antibody shows no cross-reactivity with rIL-6, rIL-6 sIL, rOFSI, rLIF, rIL-11, rhsip130 and rCNTF.

ELISA Capture - This antibody can be used as a capture antibody in a human IL-6 ELISA in combination with biotinylated, IL-6 affinity purified polyclonal detection antibody (Catalog # BAF206). A general protocol is provided on the next page. Using plates coated with 100 μg/Well of the capture antibody at 1 μg/mL in combination with 100 μg/Well of the detection antibody at 300 ng/mL, an ELISA for sample volumes of 100 μL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 2 ng/mL.

Immunohistochemistry - This antibody can be used at 0.5 - 5 μg/mL with the appropriate secondary reagents to detect human IL-6 in cultured cells or tissue sections.

Optimal dilutions should be determined by each laboratory for each application. For immunohistochemistry images, please refer to our website at http://www.RnDSystems.com/asp/c_immunohistochemistry_add.asp
**ELISA Protocol**

**Solutions Required**
- **Wash Buffer** - 0.05% Tween® 20 in PBS, pH 7.4
- **Diluent** - 0.1% BSA, 0.05% Tween 20 in Tris-buffered Saline pH 7.3 (20 mM Trizma base, 150 mM NaCl)
- **Substrate Solution** - 1:1 mixture of Color Reagent A (H₂O₂) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999)
- **Stop Solution** - 1 M H₂SO₄

**Plate Preparation**
1. Transfer 100 μL/well of the capture antibody (diluted to the appropriate concentration in PBS, use immediately) to an ELISA plate. Seal plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper toweling.
3. Block plates by adding 300 μL of PBS containing 1% BSA, 5% sucrose and 0.05% NaN₃ to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition. Alternatively, the blocking buffer can be aspirated after step 3 and the plates can be dried under vacuum. When sealed with desiccant, the plates can be stored at 4° - 8° C for at least 2 months.

**Assay Procedure**
1. Dilutions of unknowns and standards should be carried out in polypropylene tubes. Add 100 μL of sample or standards in an appropriate diluent, per well. Mix by gently tapping the plate frame for 1 minute. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 μL of the biotinylated detection antibody, diluted in the appropriate diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 μL streptavidin HRP (R&D Systems, Catalog # DY998, dilute according to the directions on the vial label) to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for 20 - 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

**Calculation of Results**
To calculate assay results, average the duplicate readings and subtract the zero standard optical density from the sample optical density. Create a standard curve using data reduction software capable of generating a four parameter (4P-L) curve fit. Alternatively, plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log-log paper and regression analysis may be applied to the log transformation. To determine the human IL-6 concentrations for each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human IL-6 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed.

**Limitations**
It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in the above protocol may be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.

A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays. The protocol