Description

The NOK-1 monoclonal antibody specifically recognizes CD178. Fas (CD95; APO-1) is a 45 kDa cell surface protein that mediates apoptosis when cross-linked with agonistic anti-Fas antibodies or by Fas ligand (FasL; CD178). Fas belongs to the TNF (Tumor Necrosis Factor)/NGF (Nerve Growth Factor) receptor family, and is expressed in various tissues and cells including the thymus, liver, ovary and lung. CD178 (FasL), a member of the TNF cytokine family, induces apoptosis by binding to Fas, its cell-surface receptor. FasL may exist as either membrane bound or soluble forms and is expressed by activated T and NK cells. FasL may also be constitutively expressed in some immunologically privileged sites, e.g., eye and testis. Fas and FasL play an important role in the induction of apoptosis, and thus regulate a variety of immunological responses.

The NOK-1 antibody clone has been reported to recognize human FasL, recognizing both the membrane bound (FasL) and soluble (sFasL) forms. It is reported that the epitope for NOK-1 has been mapped to the COOH-terminus of FasL, at the region implicated in Fas binding. FasL and sFasL have been reported to migrate at reduced molecular weights of 40 and 26 kDa, respectively. However, the molecular weights observed in a particular sample may vary according to FasL and sFasL glycosylation and breakdown patterns as described in the literature. The NOK-1 antibody clone is not recommended for the Western blot application.

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Flow cytometric analysis of CD178 (FasL) expression on FasL-transfected cells. Untransfected mouse L5178Y T lymphoma cells were cultured alone whereas human FasL-transfected L5178Y T lymphoma cells were cultured with the KB8301 metalloproteinase inhibitor for 24 hours. KB8301 blocks enzymatic cleavage of CD178 resulting in high CD178 levels to be expressed by the transfected cells. The untransfected (Left Panel) and transfected (Right Panel) cells were stained with either Purified Mouse IgG1, κ Isotype Control (Cat. No. 557273; dashed line histograms) or Purified Mouse Anti-Human CD178 (Cat. No. 556372; solid line histograms) followed by Biotin Goat Anti-Mouse Ig (Multiple Adsorption) (Cat. No. 550337) and Streptavidin-PE (Cat. No. 554061). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

Immunoprecipitation/western blot analysis of human CD178. CD178 was precipitated from human peripheral blood mononuclear cells (PBMC’s) with either Purified Mouse Anti-Human CD178 (Lane 1) or Purified Mouse IgG1, κ Isotype Control (Cat. No. 557273; Lane 2) and detected by western blot analysis with clone G247-4 (Cat. No. 556387).

The bands above and below the 40 kDa CD178 band in lane 1 and lane 2 represent the heavy and light chain of IgG used for immunoprecipitation.
Application Notes

Flow cytometry Routinely Tested
Immunoprecipitation Routinely Tested
Western blot Not Recommended

Recommended Assay Procedure:
NOK-1 has also been shown to neutralize the cytotoxic activity of FasL. Neutralization of FasL activity inhibits Fas-mediated killing. Purified NA/LE Mouse Anti-Human CD178 (Cat. No. 556371) should be used for all functional assays. NOK-1 and a related human FasL clone, NOK-2 [Cat. No. 556376 (purified) and No. 556375 (NA/LE)] may give different profiles in neutralization assays. It is thought that NOK-1 and NOK-2 likely recognize different FasL epitopes. Neither NOK-1 nor NOK-2 are suggested for western blot analysis.

Suggested Companion Products

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
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<td>Purified Mouse Anti-Human CD178</td>
<td>0.1 mg</td>
<td>G247-4</td>
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<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<td>554657</td>
<td>Stain Buffer (BSA)</td>
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<td>NOK-1</td>
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<td>556376</td>
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<td>556375</td>
<td>Purified NA/LE Mouse Anti-Human CD178</td>
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<td>NOK-2</td>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References