

## DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Selected for its ability to block the adhesion of HSB-2 cells to immobilized recombinant human (rh) ICAM-1. In direct ELISAs, approximately 5% cross-reactivity with rhICAM-3 is observed and less than 2% cross-reactivity with rhICAM-2, rhICAM-4, rhICAM-5, recombinant rat ICAM-1, recombinant mouse ICAM-1, and rhVCAM-1 is observed.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Chinese hamster ovary cell line CHO-derived recombinant human ICAM-1 Extracellular domain
<b>Endotoxin Level</b>	<0.20 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

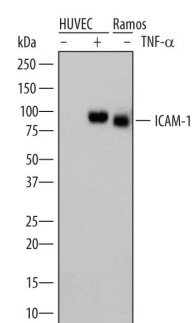
## APPLICATIONS

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
<b>Western Blot</b>	0.2 µg/mL	See Below
<b>Immunocytochemistry</b>	1-15 µg/mL	See Below
<b>Knockout Validated</b>	ICAM-1/CD54 is specifically detected in the parental Ramos human Burkitt's lymphoma cell line but is not detectable in knockout Ramos human Burkitt's lymphoma cell line.	
<b>Adhesion Blockade</b>	The adhesion of HSB2 human peripheral blood acute lymphoblastic leukemia cells ( $5 \times 10^4$ cells/well) to immobilized recombinant human ICAM-1/Fc Chimera (12.5 µg/mL, 100 µL/well) was maximally inhibited (80-100%) by 25 µg/mL of the antibody.	

## DATA

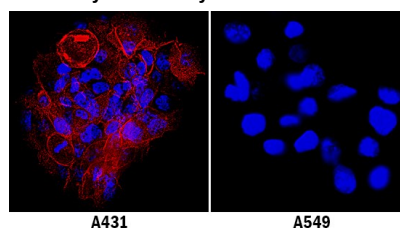
### Western Blot



### Detection of Human ICAM-1/CD54 by Western Blot.

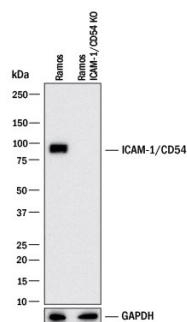
Western blot shows lysates of HUVEC human umbilical vein endothelial cells and Ramos human Burkitt's lymphoma cell line untreated (-) or treated (+) with 10 ng/mL Recombinant Human TNF-α (Catalog # [210-TA](#)) for 24 hours. PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human ICAM-1/CD54 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF720) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # [HAF016](#)). A specific band was detected for ICAM-1/CD54 at approximately 90 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

### Immunocytochemistry



**ICAM-1/CD54 in A431 Human Cell Line.** ICAM-1/CD54 was detected in immersion fixed A431 human epithelial carcinoma cell line (left panel; positive stain) and A549 human lung carcinoma cell line (right panel; negative stain) using Sheep Anti-Human ICAM-1/CD54 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF720) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # [NL010](#)) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

## Knockout Validated



**Western Blot Shows Human ICAM-1/CD54 Specificity by Using Knockout Cell Line.** Western blot shows lysates of Ramos human Burkitt's lymphoma cell line and human ICAM-1/CD54 Ramos human Burkitt's lymphoma cell line (KO). PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human ICAM-1/CD54 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF720) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ICAM-1/CD54 at approximately 90 kDa (as indicated) in the parental Ramos human Burkitt's lymphoma cell line, but is not detectable in knockout Ramos human Burkitt's lymphoma cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

Intercellular Adhesion Molecule-1 (ICAM-1) binds the leukocyte integrins LFA-1 and Mac-1. ICAM-1 expression is weak on leukocytes, epithelial and resting endothelial cells, as well as some other cell types, but expression can be stimulated by IFN-γ, TNF-α, IL-1β and LPS. Soluble ICAM-1 is found in a biologically active form in serum, probably as a result of proteolytic cleavage from the cell surface, and is elevated in patients with various inflammatory syndromes such as septic shock, LAD, cancer and transplantation.