

# **Human Ikaros Antibody**

Antigen Affinity-purified Polyclonal Goat IgG Catalog Number: AF4984

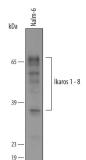
DESCRIPTION		
Species Reactivity	Human	
Specificity	Detects human Ikaros in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinat human (rh) ZIC-1, rhZNF-24, and rhZNF-206 is observed.	
Source	Polyclonal Goat IgG	
Purification	Antigen Affinity-purified	
Immunogen	E. coli-derived recombinant human Ikaros Ser427-Ser519 Accession # Q13422	
Formulation	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.	

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
Western Blot	1 μg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 μg/5 x 10 <sup>6</sup> cells	See Below
Immunocytochemistry	5-15 μg/mL	Immersion fixed Jurkat human acute T cell leukemia cell line
Intracellular Staining by Flow Cytometry	2.5 μg/10 <sup>6</sup> cells	See Below
Simple Western	10 μg/mL	See Below
CyTOF-ready	Ready to be labeled with conjugation.	I using established conjugation methods. No BSA or other carrier proteins that could interfere

## DATA

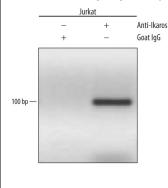
# Western Blot



### Detection of Human Ikaros by Western Blot.

Western blot shows lysates of Nalm-6 human Pre-B acute lymphocytic leukemia cell line. PVDF membrane was probed with 1  $\mu$ g/mL of Goat Anti-Human Ikaros Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4984) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). Bands were detected for Ikaros (1-8 spice forms) at approximately 37 to 63 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

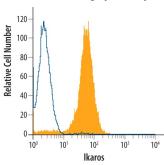
### Chromatin Immunoprecipitation (ChIP)



### Detection of Ikaros-regulated Genes by Chromatin Immunoprecipitation.

Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Ikaros/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human Ikaros Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4984) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 μL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The VPAC promoter was detected by standard PCR.

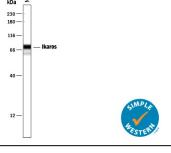
# Intracellular Staining by Flow Cytometry 120



# **Detection of Ikaros in Jurkat Human**

Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line was stained with Goat Anti-Human Ikaros Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4984, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol





**Detection of Human Ikaros by Simple** Western<sup>TM</sup>. Simple Western lane view shows lysates of Nalm-6 human Pre-B acute lymphocytic leukemia cell line, loaded at 0.2 mg/mL. Specific bands were detected for Ikaros at approximately 63-77 kDa (as indicated) using 10 µg/mL of Goat Anti-Human Ikaros Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4984) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Rev. 2/6/2018 Page 1 of 2





# **Human Ikaros Antibody**

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PREPARATION AND STORAGE			
Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.		
Shipping	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		
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  12 months from date of receipt, -20 to -70 °C as supplied.  1 month, 2 to 8 °C under sterile conditions after reconstitution.  6 months, -20 to -70 °C under sterile conditions after reconstitution.		

### BACKGROUND

Ikaros (also LyF1) is a 60 kDa member of the C2H2-type zinc-finger protein family. It is found in both T and B cells and serves as a context-dependent activator or repressor of genes. Human Ikaros is 519 amino acids (aa) in length. It possesses two zinc-finger domains, one at the N-terminus that contains four zinc-finger motifs (aa 117-224) and one at the C-terminus that contains two zinc-finger motifs (aa 462-514). The C-terminal motifs mediate homo- or heterodimerization, while the N-terminal motifs bind DNA. Multiple splice forms of 37 kDa to 47 kDa exist (Ikaros 2-8) with reduced numbers of N-terminal zinc-finger motifs. At least three are needed for DNA binding, and a heterodimer with a two-motif monomer is suggested to be transcriptionally inert. Over aa 427-519 (which are not spliced), human Ikaros shows 88% aa identity to mouse Ikaros.

Rev. 2/6/2018 Page 2 of 2

