

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse PU.1/Spi-1 in ELISAs. In direct ELISAs, no cross-reactivity with recombinant human PU.1/Spi-1 is observed. Detects mouse and rat PU.1/Spi-1 in Western Blots
Source	Monoclonal Rat IgG _{2A} Clone # 823123
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant mouse Spi-1 Met1-Lys169 Accession # P17433
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 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
Western Blot	0.1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

DATA

<p>Western Blot</p>	<p>Detection of Mouse and Rat PU.1/Spi-1 by Western Blot. Western blot shows lysates of NR8383 rat alveolar macrophage cell line and J774A.1 mouse reticulum cell sarcoma macrophage cell line. PVDF membrane was probed with 0.1 µg/mL of Rat Anti-Mouse PU.1/Spi-1 Monoclonal Antibody (Catalog # MAB7124) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for PU.1/Spi-1 at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p> <p>PU.1/Spi-1 in J774A.1 Mouse Cell Line. PU.1/Spi-1 was detected in immersion fixed J774A.1 mouse reticulum cell sarcoma macrophage cell line using Rat Anti-Mouse PU.1/Spi-1 Monoclonal Antibody (Catalog # MAB7124) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red, upper panel; Catalog # NL013) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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. <ul style="list-style-type: none"> ● 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

Spi-1 (SFV proviral integration 1 protein; also PU.1 and Sfp1) is a 37-41 kDa member of the *ets* family of transcription factors. It is both an RNA and DNA binding protein that is found in hematopoietic cells such as B cells, neutrophils, macrophages and dendritic cells (DC). Spi-1 can act as both a transcriptional activator and repressor. In DC, Spi-1 promotes the expression of CD80, CD86 and CD11b, while in proerythroblasts it blocks GATA-1 induced transcriptional activation. Spi-1 binds to DNA as a monomer and interacts with multiple factors such as Runx-1, IRF8, GATA-1 and c-Jun. Mouse Spi-1 is 272 amino acids (aa) in length. It contains a transactivation region (aa 7-117), a PEST domain (aa 118-164), and a DNA-binding domain (aa 165-266). Phosphorylation on Ser41, 142 and 148 increases activity. There is one alternative start site at Met7. Over aa 1-169, mouse Spi-1 shares 88% and 81% aa identity with rat and human Spi-1, respectively.