

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Pygopus-2 in direct ELISAs and Western blots. In direct ELISAs, no cross-reactivity with recombinant human Pygopus-1 or recombinant mouse Pygopus-1 is observed.
Source	Monoclonal Mouse IgG _{2B} Clone # 539730
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Pygopus-2 Met160-Glu336 Accession # NP_612157
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.

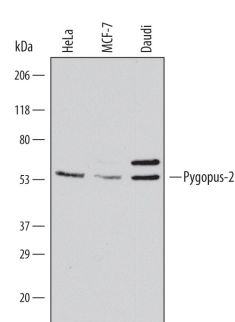
APPLICATIONS

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	0.5 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
Simple Western	5 µg/mL	See Below

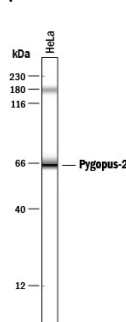
DATA

Western Blot



Detection of Human Pygopus-2 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, MCF-7 human breast cancer cell line, and Daudi human Burkitt's lymphoma cell line. PVDF Membrane was probed with 0.5 µg/mL of Mouse Anti-Human Pygopus-2 Monoclonal Antibody (Catalog # MAB3616) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Pygopus-2 at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

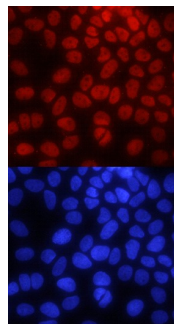
Simple Western



Detection of Human Pygopus-2 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for Pygopus-2 at approximately 65 kDa (as indicated) using 5 µg/mL of Mouse Anti-Human Pygopus-2 Monoclonal Antibody (Catalog # MAB3616). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

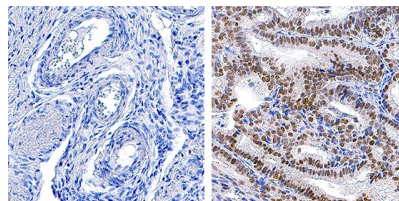


Immunocytochemistry



Pygopus-2 in MCF-7 Human Cell Line. Pygopus-2 was detected in immersion fixed MCF-7 human breast cancer cell line using Mouse Anti-Human Pygopus-2 Monoclonal Antibody (Catalog # MAB3616) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red, upper panel; Catalog # NL007) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Normal Tissue

Cancer

Pygopus-2 in Human Ovary and Ovarian Cancer Tissue. Pygopus-2 was detected in immersion fixed paraffin-embedded sections of normal human ovary (left panel) and ovarian cancer tissue (right panel) using Mouse Anti-Human Pygopus-2 Monoclonal Antibody (Catalog # MAB3616) at 3 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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

Human Pygopus-2 (pygopus; the name of a legless Australian lizard) is a 41 kDa, 406 amino acid (aa) transcriptional coactivator. It contains three domains: an N-terminal NLS (aa 41-47), followed by a proline-rich region and a zinc-finger PHD-type domain (aa 330-387). A potential internal alternate start site may generate an isoform that deletes the first 37 aa. Pygopus 2 is a nuclear protein that acts in concert with BCL-9 and TCF to retain β-Catenin in the nucleus during Wnt-signaling. It does so by forming a "chain-of-adaptors" with the above three proteins. Over the range of aa used for immunization, human pygopus 2 is 95% identical to mouse.