Human Pax6 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF8150

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Pax6 in direct ELISAs. In direct ELISAs, less than 5% cross-reactivity with recombinant human (rh) Pax1, rhPax2, rhPax3, rhPax4, rhPax5, and rhPax7 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Pax6 Met1-Arg272 Accession # P26367
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.

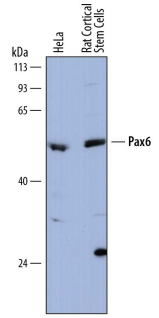
APPLICATIONS

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	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Simple Western	5 µg/mL	See Below

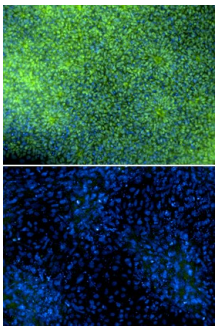
DATA

Western Blot



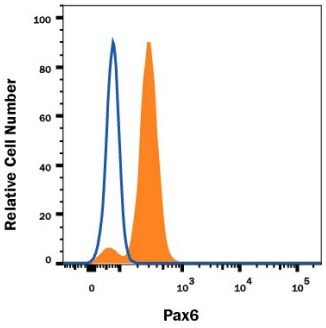
Detection of Human and Rat Pax6 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and rat cortical stem cells. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human Pax6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8150) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Pax6 at approximately 48-50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



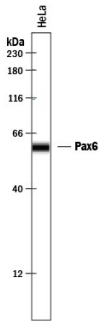
Pax6 in SA01 Human Embryonic Stem Cells. Immersion fixed SA01 human embryonic stem cells were differentiated for 6 days with Recombinant Human Noggin (Catalog # 6057-NG) and SB431542 (upper panel) or differentiated for 6 days with Recombinant Human BMP-4 (negative control, lower panel; Catalog # 314-BP). Pax6 was detected using Sheep Anti-Human Pax6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8150) at 5 µg/mL. Cells were stained using an Alexa Fluor 488-conjugated Anti-Sheep IgG Secondary Antibody (green) and counterstained with DAPI (blue). Specific staining was localized to nuclei. *Images courtesy of Dr. Ron McKay, Leiber Institute for Brain Development, Baltimore, Maryland, USA.*

Intracellular Staining by Flow Cytometry



Detection of Pax6 in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Sheep Anti-Human Pax6 Affinity-Purified Polyclonal Antibody (Catalog # AF8150, filled histogram) or Sheep IgG control Antibody (Catalog # 5-001-A, open histogram) followed by anti-Sheep IgG PE-conjugated Secondary Antibody (Catalog # F0126). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for Staining Intracellular Molecules.

Simple Western



Detection of Human Pax6 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 Pax6 at approximately 59 kDa (as indicated) using 5 µg/mL of Sheep Anti-Human Pax6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8150) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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.

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BACKGROUND

Pax6 (paired box 6; also Oculorhombin) is a 48-50 kDa member of the paired homeobox family of transcription factors. It is expressed in developing optic vesicle, olfactory dopaminergic neurons, and pancreatic endocrine cells. Pax6 is a transactivating protein that interacts with MAF, CDX2 and SOX2. Human Pax6 is 422 amino acids (aa) in length. It contains an N-terminal paired box DNA-binding domain (aa 4-130), a Gly-rich central region (aa 131-209), a homeodomain (aa 213-269) and a C-terminal Pro/Ser/Thr-rich regulatory domain (aa 279-422). Phosphorylation of the C-terminal domain at Thr281/304/373 promotes Pax6 activity. Multiple splice forms of Pax6 exist. There are alternative start sites at Met137 and a position 34 aa upstream of the standard site. There is also a deletion of aa 22-26 and 37-39, plus a 14 aa insertion after Gln47 that generates a C-terminal DNA binding site. Human and mouse Pax6 are absolutely identical in aa sequence.

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