

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat PKM2 in Western blots and detects recombinant human PKM2 in direct ELISAs.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human PKM2 Ser434-Pro531 Accession # P14618
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.

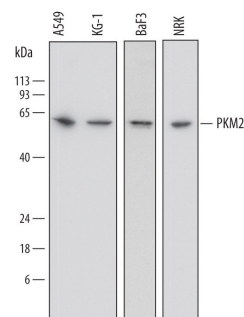
APPLICATIONS

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	0.2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	2 µg/mL	See Below
Knockout Validated	PKM2 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in PKM2 knockout HeLa cell line.	

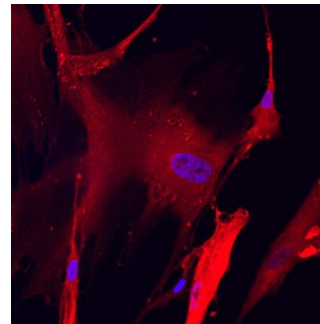
DATA

Western Blot



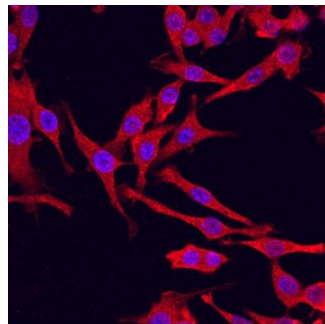
Detection of Human, Mouse, and Rat PKM2 by Western Blot. Western blot shows lysates of A549 human lung carcinoma cell line, KG-1 human acute myelogenous leukemia cell line, BaF3 mouse pro-B cell line, and NRK rat normal kidney cell line. PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for PKM2 at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



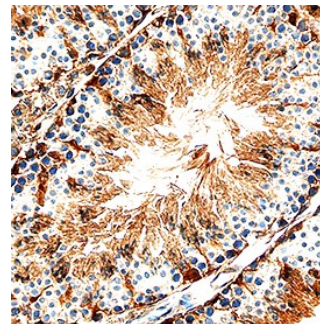
PKM2 in Wi-38 Human Cell Line. PKM2 was detected in immersion fixed Wi-38 human lung fibroblast cell line using Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



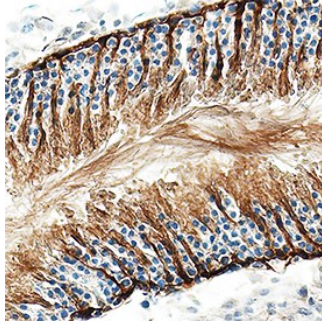
PKM2 in RAW 264.7 Mouse Cell Line. PKM2 was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cell line using Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



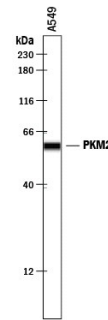
PKM2 in Mouse Testis. PKM2 was detected in perfusion fixed frozen sections of mouse testis using Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to Sertoli cells in testis. View our protocol for [Chromogenic IHC Staining of Frozen Tissue Sections](#).

Immunohistochemistry



PKM2 in Rat Testis. PKM2 was detected in perfusion fixed frozen sections of rat testis using Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to Sertoli cells in testis. View our protocol for [Chromogenic IHC Staining of Frozen Tissue Sections](#).

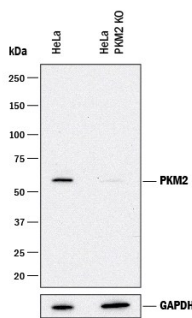
Simple Western



Detection of Human PKM2 by Simple Western™. Simple Western lane view shows lysates of A549 human lung carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for PKM2 at approximately 60 kDa (as indicated) using 2 µg/mL of Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) 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.



Knockout Validated



Western Blot Shows Human PKM2 Specificity by Using Knockout Cell Line.

Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and PKM2 knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat PKM2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7244) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for PKM2 at approximately 60 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 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

PKM2 (Pyruvate Kinase isoenzyme M2; also p58, OIP3, THBP1 and CTHBP) is a 58-60 kDa member of the PK family of enzymes. It is widely expressed, being found both intracellularly and in blood, and represents the more common splice variant of the PKM gene. PKM2 generates ATP and pyruvate by catalyzing the transfer of a phosphoryl group from PEP to ADP. Thus, when active, PKM2 promotes energy production and glycolysis. PKM2 exists as a marginally active monomer, with full activity achieved through homotetramerization. Notably, in tumor cells, select oncogenes appear to induce PKM2 homodimerization which limits PKM2 activity. PKM2 is known to be regulated by the binding of T3 and Fru-1,6-bisP. Human PKM2 is 531 amino acids (aa) in length. It contains a catalytic region (aa 43-527) plus four utilized Ser/Thr and Tyr phosphorylation sites, respectively. PKM1 is another PKM gene splice variant that shows a 45 aa substitution for aa 389-433 of PKM2. This variant shows limited expression (striated muscle) and hyperbolic Michaelis-Menten kinetics. There are additional isoform variants of PKM2 that show either a deletion of aa 59-132, or a 67 aa substitution for aa 1-82. Over aa 434-531, human PKM2 shares 95% aa sequence identity with mouse PKM2.