

DESCRIPTION

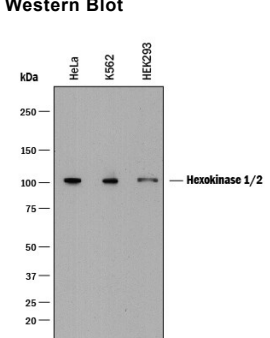
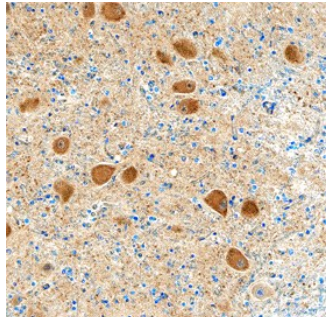
Species Reactivity	Human
Specificity	Detects human Hexokinase 1 and human Hexokinase 2 in direct ELISAs and Western blots.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Hexokinase 1 and <i>E. coli</i> -derived recombinant human Hexokinase 2 Phe11-Ser917 and Phe11-Arg917 Accession # P19367 & P52789
Formulation	Supplied as a solution in PBS containing BSA, Glycerol and Sodium Azide. See Certificate of Analysis for details. 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	1:1000 dilution	See Below
Immunohistochemistry	1:200 dilution	See Below

DATA

Western Blot	Immunohistochemistry
 <p>Detection of Human Hexokinase 1/2 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, K562 human chronic myelogenous leukemia cell line, and HEK293 human embryonic kidney cell line. PVDF membrane was probed with 1:1000 dilution of Rabbit Anti-Human Hexokinase 1/2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8178) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Hexokinase 1/2 at approximately 105 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	 <p>Hexokinase 1/2 in Human Brain Medulla Tissue. Hexokinase 1/2 was detected in immersion fixed paraffin-embedded sections of human brain medulla tissue using Rabbit Anti-Human Hexokinase 1/2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8178) at 1:200 dilution overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). Specific staining was localized to neurons. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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 opening. • 6 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Hexokinases phosphorylate hexose to form hexose 6-phosphate, the first step in hexose metabolism (1). Phosphorylation of a hexose adds charge to molecule thereby making it difficult to transport out of a cell. The hexose is therefore retained for intracellular metabolic processes, such as glycolysis or glycogen synthesis. In most organisms, glucose is the most important substrate of hexokinases and glucose-6-phosphate is the most important product. There are four mammalian hexokinases (2). Hexokinase 1, 2 and 3 are referred to as high-affinity hexokinases because their K_m for glucose is below 1 mM. Hexokinase 4 is specific for glucose and is also referred to as glucokinase (3). Hexokinase I localizes to the outer membrane of mitochondria and is found in all mammalian tissues. The amino acids involved in mitochondrion membrane localization (4) have been removed in the recombinant enzyme. Hexokinase 1 (HK1) contains two homologous halves that are believed to be evolved from a single ancestral hexokinase by gene duplication and fusion (5). While the regulatory function is associated with the N-terminal half, the catalytic site is associated with the C-terminal half. HK1 is insensitive to product inhibition (6). Mutation in the active site of human hexokinase is associated with hexokinase deficiency and severe nonspherocytic hemolytic anemia (7). The enzymatic activity of recombinant human HK1 is measured using a phosphatase-coupled method (8).

References:

1. Aleshin, A.E. et al. (1998) *Structure* 6:39-50
2. Takeda, J. et al. (1993) *J. Biol. Chem.* 268:15200.
3. Lange, A.J. et al. (1991) *Biochem. J.* 277:159-163.
4. Magnani, M. et al. *J. Biol. Chem.* 266: 502.
5. Nishi, S. et al. (1988) *Biochem. Biophys. Res. Commun.* 157:937.
6. Magnani, M. et al. (1992) *Biochem. J.* 285:193.
7. Van Wijk R. et al. (2003) *Blood* 101:345.
8. Wu, Z.L. (2011) *PLoS One* 6:e23172.

PRODUCT SPECIFIC NOTICES

* Contains <0.1% Sodium Azide, which is not hazardous at this concentration according to GHS classifications. Refer to SDS for additional information and handling instructions.

