

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Hexokinase 2 in direct ELISAs. Detects human Hexokinase 1 and human Hexokinase 2 in Western blots.
Source	Monoclonal Mouse IgG ₁ Clone # 927312
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Hexokinase 2. Phe11-Arg917 Accession # P52789
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.

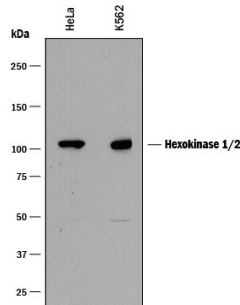
APPLICATIONS

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

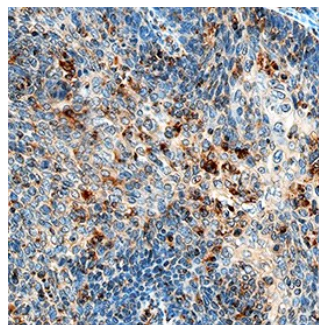
DATA

Western Blot



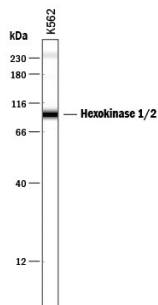
Detection of Human Hexokinase 1/2 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 0.2 µg/mL of Mouse Anti-Human Hexokinase 1/2 Monoclonal Antibody (Catalog # MAB8179) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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.

Immunohistochemistry



Hexokinase 1/2 in Human Cervical Cancer Tissue. Hexokinase 1/2 was detected in immersion fixed paraffin-embedded sections of human cervical cancer tissue using Mouse Anti-Human Hexokinase 1/2 Monoclonal Antibody (Catalog # MAB8179) at 15 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 cytoplasm in cancer cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Simple Western



Detection of Human Hexokinase 1/2 by Simple Western™. Simple Western lane view shows lysates of K562 human chronic myelogenous leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Hexokinase 1/2 at approximately 96 kDa (as indicated) using 5 µg/mL of Mouse Anti-Human Hexokinase 1/2 Monoclonal Antibody (Catalog # MAB8179). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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	<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

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 2 (HK2), also known as muscle form hexokinase, localizes to the outer membrane of mitochondria and is present in adipose tissue, skeletal muscle, and heart (4). The amino acids corresponding to the mitochondrial binding domain (5) have been removed in the recombinant enzyme. Like Hexokinase 1 (HK1), HK2 contains two homologous halves that may have evolved from an ancestral hexokinase through gene duplication and tandem ligation (6). Unlike HK1, in HK2 both the C-terminal and N-terminal portions are catalytically active with the N-terminal half having higher activity than the C-terminal half (7). In HK2 both the N-terminal and C-terminal halves exhibit product inhibition. HK2 overexpression is required for tumor growth making HK2 an attractive oncotarget (4). The enzymatic activity of recombinant human HK2 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.
4. Patra, K.C. *et al.* (2013) *Cancer Cell.* **24**:213.
5. Bianchi M *et al.* (1998) *Mol Cell Biochem* **189**:185.
6. Ureta, T. (1982) *Comp Biochem Physiol. B.* **71B**:549.
7. Ahn, K.J. *et al.* (2009) *BMB Rep.* **42**:350.
8. Wu, Z.L. (2011) *PLoS One* **6**:e23172.