ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Pyruvate Kinase M1/M2 (OIP3; PK3; PKM1) Antibodies

Cat # PKM14-P	Human PK-M1/M2 Control/Blocking Peptide	**SIZE**: 100 µg
Cat # PKM14-A	Rabbit anti-Human PK-M1/M2 IgG (affinity pure)	**SIZE**: 100 µg

Pyruvate kinase is an enzyme involved in glycolysis. It catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate to ADP, yielding a pyruvate molecule. There are 4 isozymes of pyruvate kinase in mammals: L, R, M1 and M2. L type is major isozyme in the liver, R is found in red cells, M1 is the main form in muscle, heart and brain, and M2 is found in early fetal tissues as well as in most cancer cells.

Human PKM1/2 is 531 aa protein. Human PKM2 389-433 aa (IYHLQLFEELRR LAPITSDPTE ATAVGAVEASFKCCSGAIIVLTK) is replaced by (MFHRKLFEELVRASSHSTDLMEAMA MGSVEASYKCLAAAL IVLTE) in PKM1. The PKM2 isoenzyme of pyruvate kinase is specifically expressed at high levels in tumor cells, and can be measured in plasma of patients with advanced breast cancer. The marker is useful for measuring disease activity, sensitivity to chemotherapy and recurrence.

Synonyms:
Pyruvate kinase isozymes M1/M2, EC 2.7.1.40, Pyruvate kinase muscle isozyme, Pyruvate kinase 2/3, Cytosolic thyroid hormone-binding protein, CTHBP, THBP1, M2PK, PKM2, PK3, PK2, PKM, TCB, OIP3, MGC3932, Tumor Type M2 Pyruvate Kinase

Source of Antigen and Antibodies

Antigen	16-aa peptide of Human PK-M1/M2 (Protein accession # (P14618); ref. 1); designated as PKM14-P control/blocking peptide conjugated to KLH; epitope location ~ N-terminus
Antibody host/type	Rabbit, Polyclonal IgG (Cat # PKM14-A), purified over antigen-Agarose
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control Ab	Non-immune rabbit IgG (Cat # 20009-1) to be used as –ve control for ELISA, WB, IHC.

Human PKM2 (~62 kda) was expressed in E. coli as his-tag and purified (>95%). For Western blot +ve control (**Cat # PKM14-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **PKM14**-C for good visibility with antibody Cat # **PKM14**-A. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **PKM14**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG

100 ug/100ul solution lyophilized powder
Supplied in Buffer: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide

100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Long-term: At –20^{0}C or below in suitable aliquots after reconstitution. Do not freeze and thaw working and dilute solutions.

Stability: 6-32 months at –20^{0}C or below.

Shipping: 4^{0}C for solutions and room temp for powder.

Recommended Usage

Western Blotting: 1-10 µg/ml; using affinity pure antibody (chemiluminescence technique).

ELISA: 1:100K; using 50-100 ng control peptide/well.

Histochemistry & Immunofluorescence: Not tested; we recommend the use of affinity purified antibody at 2-10 µg/ml.

Specificity & Cross-reactivity

Human PKM14-P peptide sequence is located near the N-terminus of PKM1/2. This is conserved in PKM1 and M2 isoform. Antibody cross-reactivity in various species is not known. The control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-30 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at the web site).

General References: (1) Tani K, et al., (1988) Proc. Natl. Acad. Sci. U.S.A. 85:1792-1795; Takenaka M. et al., (1991) Eur. J. Biochem. 198:101-106

*This product is for in vitro research use only.

Related material available from ADI

Antibodies to all forms of Pyruate Kinases

PKM14-A-P-C 80122A

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