Revision 3

#5759 Store at -20C

Phospho-AMPK Substrate Motif [LXRXX(pS/pT) MultiMab® Rabbit mAb mix

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source/Isotype:
W, IP, E-P	All	Endogenous	Rabbit

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Peptide ELISA (DELFIA)	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-AMPK Substrate Motif [LXRXX(pS/pT) MultiMab® Rabbit mAb mix preferentially recognizes endogenous proteins and peptides bearing the LXRXXpS/pT motif. The antibody also cross-reacts with proteins and peptides that only harbor an RXXpS/pT motif.

Source / Purification

MultiMab® rabbit monoclonal mix antibodies are prepared by combining individual rabbit monoclonal clones in optimized ratios for the approved applications. Each antibody in the mix is carefully selected based on motif recognition and performance in multiple assays. Each mix is engineered to yield the broadest possible coverage of the modification being studied while ensuring a high degree of specificity for the modification or motif.

Background

AMP-activated protein kinase (AMPK) is highly conserved from yeast to plants and animals and plays a key role in the regulation of energy homeostasis (1). AMPK is a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3) (2). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia (1). The tumor suppressor LKB1, in association with accessory proteins STRAD and MO25, phosphorylates AMPKα at Thr172 in the activation loop, and this phosphorylation is required for AMPK activation (3-5). AMPKα is also phosphorylated at Thr258 and Ser485 (for α1; Ser491 for α2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The β1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101, Ser108, and Ser182 (6,7). Phosphorylation at Ser108 of the β1 subunit seems to be required for AMPK activation, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). Several mutations in AMPKγ subunits have been identified, most of which are located in the putative AMP/ATP binding sites (CBS or Bateman domains). Mutations at these sites lead to reduction of AMPK activity and cause glycogen accumulation in heart or skeletal muscle (1,2). Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS (1).~AMPK phosphorylates consensus motif (L/M)XRXX(S/T)XXXL (8). Antibodies recognizing the LXRXX(S/T) motif are very useful in the identification of AMPK substrates.

Background References

1. Hardie, D.G. (2004) *J Cell Sci* 117, 5479-87.
2. Carling, D. (2004) *Trends Biochem Sci* 29, 18-24.
3. Hawley, S.A. et al. (1996) *J Biol Chem* 271, 27879-87.
4. Lizcano, J.M. et al. (2004) *EMBO J* 23, 833-43.
5. Shaw, R.J. et al. (2004) *Proc Natl Acad Sci USA* 101, 3329-35.
6. Woods, A. et al. (2003) *J Biol Chem* 278, 28434-42.
7. Warden, S.M. et al. (2001) *Biochem J* 354, 275-83.
8. Gwinn, D.M. et al. (2008) *Mol Cell* 30, 214-26.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **E-P:** Peptide ELISA (DELFIA)

Cross-Reactivity Key

All: All Species Expected

Trademarks and Patents

Limited Uses