## Phylogeny

ALPK3 is a member of the atypical alpha-kinase (α-kinase) family, which represents an exceptional branch on the protein kinase tree with low sequence homology to conventional eukaryotic protein kinases (mcnamara2023alphakinase3 pages 1-2, li2023anovelcompound pages 11-11, middelbeek2010thealphakinasefamily pages 1-2). The family is considered an evolutionarily recent branch limited to eukaryotes and is widely distributed among vertebrates (middelbeek2010thealphakinasefamily pages 1-2). The human alpha-kinase family includes six members: ALPK1, ALPK2, ALPK3, eEF2K, TRPM6, and TRPM7 (cheawsamoot2023investigationofalpha pages 26-32). ALPK3 is most closely evolutionarily related to ALPK2, with which it shares an identical domain architecture (cheawsamoot2023investigationofalpha pages 26-32). It is classified within this family based on kinome analyses by Manning et al. (mcnamara2023alphakinase3 pages 1-2, wang2024spectrumandgenotype–phenotype pages 7-7).

## Reaction Catalyzed

There is conflicting information regarding the catalytic activity of ALPK3. Some sources state that it is an alpha-type serine/threonine protein kinase that catalyzes phosphorylation reactions, transferring a phosphate group from ATP to protein substrates (mcnamara2023alphakinase3 pages 1-2, li2023anovelcompound pages 11-11, wang2024spectrumandgenotype–phenotype pages 7-7, unknownauthors2021theroleof pages 1-12).

Conversely, other studies report that ALPK3 functions biologically as a pseudokinase, lacking detectable kinase activity (feng2023alpk3functionsas pages 1-3, andrade2025exploringtherole pages 5-6, leinhos2025analpk3truncation pages 21-31). This is supported by in vitro assays with the murine ALPK3 catalytic domain and in vivo studies where knock-in mice with a K1420R mutation in the catalytic lysine residue maintain normal cardiac function (feng2023alpk3functionsas pages 1-3, unknownauthors2021theroleof pages 12-16). Furthermore, phosphoproteomic analyses of mutant ALPK3 mouse hearts and iPSC-derived cardiomyocytes showed no significant changes in phosphorylation (feng2023alpk3functionsas pages 1-3, cheawsamoot2023investigationofalpha pages 32-36).

## Cofactor Requirements

Information on cofactor requirements is contradictory, reflecting the debate on ALPK3’s catalytic activity. Sources describing it as an active kinase state that it requires Mg²⁺ as a cofactor, which is typical for the alpha-kinase family (wang2024spectrumandgenotype–phenotype pages 7-7, middelbeek2010thealphakinasefamily pages 1-2). In contrast, sources classifying ALPK3 as a pseudokinase state that cofactor requirements are absent or that there is no definitive evidence for them (feng2023alpk3functionsas pages 1-3, andrade2025exploringtherole pages 5-6).

## Substrate Specificity

A comprehensive atlas of substrate specificities for the human serine/threonine kinome provided consensus substrate specificity data for ALPK3, derived from peptide library screening and presented as position-specific scoring matrices (johnson2023anatlasof pages 9-10). The consensus substrate motif for ALPK3 reflects the distinct specificity of the alpha-kinase family (johnson2023anatlasof pages 4-4). The alpha-kinase family is characterized by its ability to phosphorylate serine and threonine residues located within α-helices, differing from conventional kinases (mcnamara2023alphakinase3 pages 1-2, cheawsamoot2023investigationofalpha pages 26-32). ALPK3 is described as having likely conserved substrate specificity for stromal sarcomeric proteins and preferentially phosphorylating cardiac structural proteins (mcnamara2023alphakinase3 pages 1-2, unknownauthors2021theroleof pages 1-12). However, studies supporting its pseudokinase function found no changes in phosphorylation of known substrates like SQSTM1 in kinase-dead mouse models (feng2023alpk3functionsas pages 1-3).

## Structure

Detailed 3D structural data for ALPK3 are limited (li2023anovelcompound pages 11-11, andrade2025exploringtherole pages 5-6). The protein’s domain organization includes N-terminal and C-terminal immunoglobulin-like (Ig-like) domains that flank a C-terminal alpha-kinase domain (cheawsamoot2023investigationofalpha pages 26-32, unknownauthors2021theroleof pages 41-48). A large intrinsically disordered region (IDR) is located between the Ig-like domains (unknownauthors2021theroleof pages 41-48). The alpha-kinase catalytic domain possesses a unique kinase fold distinct from canonical eukaryotic protein kinases (johnson2023anatlasof pages 4-4, unknownauthors2021theroleof pages 1-12). The alpha-kinase domain of ALPK3 is characterized by unique structural features, including a noncanonical activation loop and C-helix conformation, as well as distinctive hydrophobic spine arrangements (johnson2023anatlasof pages 4-4). Despite sequence homology and conservation of the invariant catalytic lysine residue (K1420), a 3D structural alignment with the confirmed alpha-kinase TRPM7 showed divergence in other residues affecting catalytic activity, supporting its classification as a pseudokinase (feng2023alpk3functionsas pages 1-3).

## Regulation

The regulatory mechanisms of ALPK3 are not distinctly described and remain incompletely characterized (andrade2025exploringtherole pages 5-6, cheawsamoot2023investigationofalpha pages 26-32, cheawsamoot2023investigationofalpha pages 32-36). It is suggested that regulation involves post-translational modifications (PTMs) such as phosphorylation that modulate kinase activity, consistent with general protein kinase regulatory paradigms (li2023anovelcompound pages 11-11, wang2024spectrumandgenotype–phenotype pages 7-7, unknownauthors2021theroleof pages 1-12). However, one study makes no direct mention of PTMs regulating ALPK3 itself (mcnamara2023alphakinase3 pages 1-2). The intrinsically disordered region (IDR) in ALPK3 is predicted to undergo phase separation to form biomolecular condensates, which may serve as regulatory hubs (unknownauthors2021theroleof pages 41-48).

## Function

ALPK3 is predominantly expressed in cardiac and skeletal muscle, with the highest expression noted in cardiomyocytes (feng2023alpk3functionsas pages 1-3, mcnamara2023alphakinase3 pages 1-2, cheawsamoot2023investigationofalpha pages 26-32). Its subcellular localization is debated, with reports of localization to the M-band of the sarcomere, the nuclear envelope, and a predominant presence in the nucleus (mcnamara2023alphakinase3 pages 1-2, andrade2025exploringtherole pages 5-6, cheawsamoot2023investigationofalpha pages 26-32, unknownauthors2021theroleof pages 12-16). ALPK3 is essential for cardiac development and function, maintaining sarcomere integrity and muscle proteostasis (feng2023alpk3functionsas pages 1-3, mcnamara2023alphakinase3 pages 1-2). It plays a regulatory role in the expression and positioning of myomesins (MYOM1 and MYOM2) (andrade2025exploringtherole pages 5-6). Interacting partners include sarcomeric proteins like titin, myomesins, TMOD1, MYH6, MYH7, and MYH9, as well as the ubiquitin-binding protein SQSTM1 (p62) (mcnamara2023alphakinase3 pages 1-2, unknownauthors2021theroleof pages 34-41, andrade2025exploringtherole pages 5-6). It also interacts with nuclear proteins such as HMGB2, DDX3X, and HIST1H1E (unknownauthors2021theroleof pages 34-41). Its function may be independent of kinase activity and mediated by protein-protein interactions as a scaffold (unknownauthors2021theroleof pages 12-16, unknownauthors2021theroleof pages 41-48).

## Other Comments

Biallelic truncating and compound heterozygous variants in ALPK3 cause severe pediatric hypertrophic and dilated cardiomyopathies, often with associated skeletal and craniofacial features (li2023anovelcompound pages 11-11, cheawsamoot2023investigationofalpha pages 26-32, unknownauthors2021theroleof pages 12-16). Monoallelic truncating variants are associated with adult-onset hypertrophic cardiomyopathy (cheawsamoot2023investigationofalpha pages 26-32). The pathogenic mechanism is loss-of-function, with variants disrupting sarcomeric organization and leading to contractile dysfunction (mcnamara2023alphakinase3 pages 1-2, cheawsamoot2023investigationofalpha pages 26-32). The pathogenicity of ALPK3 variants is likely due to effects on protein levels, interacting partners, or subcellular localization, rather than on kinase activity (feng2023alpk3functionsas pages 1-3). This is supported by mouse models, where *Alpk3* knockout leads to dilated cardiomyopathy, but a knock-in of the kinase-dead K1420R mutation does not cause cardiac defects (feng2023alpk3functionsas pages 1-3, unknownauthors2021theroleof pages 12-16).

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