1. Phylogeny  
   PSKH2 (Serine/threonine‐protein kinase H2, UniProt: Q96QS6) is classified within the non‐specific serine/threonine protein kinase family (EC 2.7.11.1), a group of enzymes conserved across eukaryotic species that catalyze phosphorylation on serine or threonine residues (anti2009nonspecificserinethreonineprotein pages 1-7). PSKH2 shares a high degree of sequence similarity with its paralog PSKH1, and while PSKH1 is catalytically active, PSKH2 is considered to be a pseudokinase in that it lacks detectable catalytic activity (horne2025pskh1kinaseactivity pages 5-6). Comparative genomic analyses of non‐specific serine/threonine kinases indicate that orthologs of PSKH2 are likely to be present in other mammals, reflecting an evolutionary conservation of key structural domains critical for kinase function (anti2009nonspecificserinethreonineprotein pages 16-19). The phylogenetic placement of PSKH2 is supported by extensive analyses of kinase complements in multiple species, which demonstrate that the non‐specific serine/threonine protein kinases constitute an ancient family that can be traced back to early eukaryotic evolution (anti2009nonspecificserinethreonineprotein pages 29-32). In addition, the high sequence identity with PSKH1 suggests that PSKH2 emerged via gene duplication early in the evolution of the human kinome and may retain regulatory or scaffolding roles despite its lack of catalytic turnover (horne2025pskh1kinaseactivity pages 5-6). Detailed conservation of the catalytic domain among members of this family, including the glycine‐rich loop, DFG motif, and activation segment, further supports an evolutionary relationship among these kinases, with PSKH2 representing a divergent branch that has lost classical catalytic function (anti2009nonspecificserinethreonineprotein pages 25-27).
2. Reaction Catalyzed  
   The enzymatic reaction catalyzed by serine/threonine kinases follows the general reaction: ATP + protein–(L‐serine or L‐threonine) → ADP + protein–(L‐serine/threonine)‐phosphate + H^+ (anti2009nonspecificserinethreonineprotein pages 1-7). In principle, PSKH2 would be expected to mediate such a phosphoryl transfer if it were catalytically active; the reaction involves a nucleophilic attack by the hydroxyl group of a serine or threonine residue on the γ‐phosphate of ATP (anti2009nonspecificserinethreonineprotein pages 42-45). This canonical kinase reaction is conserved among non‐specific serine/threonine kinases and is a central feature of their role in signal transduction and protein regulation (anti2009nonspecificserinethreonineprotein pages 42-45). Despite the similarity in the catalytic domain to other kinases, PSKH2 is classified as a pseudokinase, and therefore its ability to catalyze this reaction under physiological conditions has not been demonstrably confirmed (horne2025pskh1kinaseactivity pages 5-6).
3. Cofactor Requirements  
   As with most members of the serine/threonine kinase family, the catalytic activity of PSKH2, if present, would be expected to depend on divalent metal ion cofactors, particularly Mg^2+, which is necessary for the stabilization of ATP within the active site (anti2009nonspecificserinethreonineprotein pages 7-10). The Mg^2+ ion functions by coordinating to the phosphate groups of ATP and facilitating the correct orientation of the nucleotide for phosphotransfer (anti2009nonspecificserinethreonineprotein pages 7-10). In addition, a weak contribution from Mn^2+ has been reported for related kinases in some experimental systems, although Mg^2+ remains the primary cofactor (anti2009nonspecificserinethreonineprotein pages 7-10). Consequently, the cofactor requirement of PSKH2 is consistent with that of other non‐specific serine/threonine protein kinases even though its intrinsic kinase activity is in question (anti2009nonspecificserinethreonineprotein pages 7-10).
4. Substrate Specificity  
   Studies of non‐specific serine/threonine kinases have shown that many family members exhibit broad substrate specificity, often phosphorylating a wide range of protein targets that contain serine or threonine residues within flexible sequence motifs (anti2009nonspecificserinethreonineprotein pages 25-27). Although PSKH2-specific substrate details have not been explicitly delineated, it is anticipated that its substrate specificity would mirror that of related kinases, which typically target sequences enriched in basic and hydrophobic residues surrounding the phosphoacceptor site (anti2009nonspecificserinethreonineprotein pages 34-37). For example, related kinases within this family have been reported to favor motifs where serine or threonine is preceded by one or more arginine or lysine residues and followed by hydrophobic amino acids, thereby creating a docking site that positions the substrate optimally for phosphorylation (anti2009nonspecificserinethreonineprotein pages 25-27, anti2009nonspecificserinethreonineprotein pages 34-37). It should be noted that PSKH2’s classification as a pseudokinase implies that its engagement with substrates, if any, could occur through non-catalytic modes such as scaffolding or allosteric modulation rather than through active phosphoryl transfer (anti2009nonspecificserinethreonineprotein pages 51-54).
5. Structure  
   The three-dimensional structure of PSKH2 is predicted to comprise the characteristic bilobal architecture common to serine/threonine protein kinases, with an N-terminal lobe predominantly organized into β-sheets and a larger C-terminal lobe composed mainly of α-helices (anti2009nonspecificserinethreonineprotein pages 109-111). The central kinase domain is expected to house critical structural motifs such as the glycine-rich loop—which contributes to ATP binding—and the conserved DFG motif that is essential for coordinating Mg^2+ binding to ATP (anti2009nonspecificserinethreonineprotein pages 42-45). The activation loop, located within the C-terminal lobe, typically undergoes phosphorylation in catalytically active kinases to stabilize the active conformation; however, in PSKH2, sequence analysis indicates that alterations in this region may underlie its lack of catalytic activity (horne2025pskh1kinaseactivity pages 5-6). In addition, the structure likely features a C-helix, which is critical in positioning catalytic residues for effective phosphoryl transfer in active kinases, and a hydrophobic spine that maintains the structural integrity of the active site (anti2009nonspecificserinethreonineprotein pages 109-111). Although definitive experimental structures, such as those derived from X-ray crystallography or cryo-electron microscopy, have not been published specifically for PSKH2, computational models based on AlphaFold and homology modeling from related kinases provide a reasonable approximation of its domain organization and overall tertiary structure (anti2009nonspecificserinethreonineprotein pages 29-32, anti2009nonspecificserinethreonineprotein pages 64-67). The pseudokinase status of PSKH2 is further supported by subtle sequence deviations in key catalytic residues that are indispensable for phosphotransfer activity, a feature that is consistent with the observation that PSKH2 retains high sequence similarity to PSKH1 yet lacks demonstrable kinase activity (horne2025pskh1kinaseactivity pages 5-6). Furthermore, the regulatory regions flanking the central kinase domain—often characterized by intrinsically disordered segments—could provide interaction interfaces for other proteins, thereby enabling PSKH2 to function as a modulatory scaffold despite its inactive catalytic core (anti2009nonspecificserinethreonineprotein pages 29-32). Structural comparisons with other non-specific serine/threonine kinases reveal that these flanking regions may contribute to subcellular localization and the assembly of multiprotein complexes, functions that are likely conserved in PSKH2 (anti2009nonspecificserinethreonineprotein pages 64-67, anti2009nonspecificserinethreonineprotein pages 109-111).
6. Regulation  
   The regulation of serine/threonine kinases generally involves a multitude of mechanisms including autophosphorylation, phosphorylation by upstream activators, and allosteric modulation via binding to regulatory proteins, and these principles are broadly applicable to the kinase family encompassing PSKH2 (anti2009nonspecificserinethreonineprotein pages 45-47). In catalytically active kinases, phosphorylation typically occurs on residues within the activation loop, thereby inducing conformational changes that promote full enzymatic activity; however, PSKH2 is noted to be catalytically inactive, suggesting that its regulation is predominantly mediated through alternative mechanisms such as protein–protein interactions or allosteric effects (horne2025pskh1kinaseactivity pages 5-6). Studies of PSKH1 have demonstrated that Ca^2+ sensor proteins, including calmodulin and certain CREC family members, differentially modulate its kinase activity via direct binding to the kinase domain, and given the high sequence identity between PSKH1 and PSKH2, similar regulatory interactions may influence PSKH2 function even in the absence of catalytic activity (horne2025pskh1kinaseactivity pages 5-6, anti2009nonspecificserinethreonineprotein pages 114-116). No specific post-translational modification sites have been definitively mapped for PSKH2, and thus the contributions of phosphorylation, ubiquitination, or other modifications to its regulation remain to be fully elucidated (anti2009nonspecificserinethreonineprotein pages 116-119). In kinases where regulatory phosphorylation is critical, specific residues within the activation segment are modified by upstream kinases such as PDK1 or mTORC1; however, for PSKH2, the absence of measurable catalytic activity implies that its role may be confined to serving as a docking platform or an allosteric regulator in larger signaling complexes rather than directly transferring phosphate groups (horne2025pskh1kinaseactivity pages 5-6). Moreover, conformational dynamics driven by interactions with other proteins could govern the subcellular localization or stability of PSKH2, thereby indirectly influencing cellular signaling networks (anti2009nonspecificserinethreonineprotein pages 114-116). Overall, while classical enzymatic regulation via phosphorylation is well established for many serine/threonine kinases, PSKH2 is primarily regulated through mechanisms that remain to be characterized in detail and are likely to involve non-catalytic functions (anti2009nonspecificserinethreonineprotein pages 116-119).
7. Function  
   The biological function of PSKH2 remains to be fully elucidated, and current literature provides only indirect insights into its potential roles, primarily inferred from its membership in the non-specific serine/threonine kinase family (anti2009nonspecificserinethreonineprotein pages 87-89). Proteins within this kinase family are known to participate in a wide array of cellular processes including cell cycle regulation, signal transduction, apoptosis, and the maintenance of cellular homeostasis through the regulation of protein phosphorylation (anti2009nonspecificserinethreonineprotein pages 25-27). Although PSKH2 has been defined as a pseudokinase, its high sequence conservation with the active kinase PSKH1 suggests that it may contribute to cellular signaling through scaffolding functions or by modulating the assembly of higher-order protein complexes (horne2025pskh1kinaseactivity pages 5-6). In other well-characterized kinases, active phosphorylation events are critical for propagating signals that regulate mRNA transcription, protein translation, and cytoskeletal dynamics; by analogy, PSKH2 may influence such processes by serving as a regulatory adaptor or by competing with active kinases for substrate or effector binding (anti2009nonspecificserinethreonineprotein pages 29-32). There is also evidence from studies of related kinases that perturbations in kinase activity or alterations in subcellular localization can have significant physiological effects, such as those observed in kidney ciliopathies which have been linked to defects in PSKH1 function (horne2025pskh1kinaseactivity pages 5-6). Although specific substrates or interacting partners of PSKH2 have not been conclusively identified, its evolutionary conservation and the maintenance of a complete kinase fold imply that PSKH2 might play a role in modulating signaling pathways by acting as a molecular scaffold, thereby organizing spatially and temporally distinct signaling modules within the cell (anti2009nonspecificserinethreonineprotein pages 51-54). In addition, members of the non-specific serine/threonine kinase family are frequently involved in stress response pathways, suggesting that PSKH2 could participate in cellular adaptations to environmental or developmental stimuli (anti2009nonspecificserinethreonineprotein pages 92-94). Accordingly, although the direct functional outputs of PSKH2 remain under active investigation, its involvement in key cellular pathways is supported by the regulatory themes common to the broader kinase family (anti2009nonspecificserinethreonineprotein pages 25-27).
8. Other Comments  
   At present, there are no specific small-molecule inhibitors that selectively target PSKH2, and the pseudokinase nature of PSKH2 implies that traditional ATP-competitive inhibitors used against catalytically active kinases may not be effective (anti2009nonspecificserinethreonineprotein pages 119-121). More broadly, the development of chemical probes for pseudokinases remains challenging due to the absence of measurable catalytic activity, and researchers have yet to establish whether PSKH2 can be modulated pharmacologically to alter its putative scaffolding or regulatory roles (anti2009nonspecificserinethreonineprotein pages 119-121). In terms of disease association, while dysregulation of serine/threonine kinases is a common feature in various pathologies such as cancer, neurodegenerative diseases, and ciliopathies, there are currently no definitive reports linking PSKH2 directly to any specific human disorders (anti2009nonspecificserinethreonineprotein pages 97-99). Notably, mutations in the functionally active paralog PSKH1 have been associated with kidney ciliopathies, yet analogous genetic alterations in PSKH2 have not been conclusively identified, leaving its clinical relevance an open question (horne2025pskh1kinaseactivity pages 5-6). Ongoing research into the human kinome continues to explore the functional roles of pseudokinases like PSKH2, and future studies may reveal novel mechanisms of cellular regulation that involve non-catalytic functions such as molecular scaffolding, subcellular targeting, or allosteric modulation of adjacent kinase activities (anti2009nonspecificserinethreonineprotein pages 25-27). In summary, while the enzymatic and structural properties of PSKH2 have been inferred from studies on related kinases, further experimental work is required to fully delineate its role in intracellular signaling and potential contributions to human disease (horne2025pskh1kinaseactivity pages 5-6).
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