1. Phylogeny  
   RIPK4 (Receptor-interacting serine/threonine-protein kinase 4), also known as ANKRD3 or DIK, is a member of the serine/threonine kinase superfamily that emerged as part of the receptor-interacting protein kinase (RIPK) grouping. Comparative genomic analyses, as described in seminal works on the human kinome, place RIPK4 within a distinct subgroup often classified in the RIPK/WNK cluster, which is evolutionarily related to other receptor‐interacting protein kinases and shares motif–selectivity characteristics with these kinases (johnson2023anatlasof pages 2-3, krupa2002therepertoireof pages 1-2). Phylogenetic studies using sequence similarity and domain architecture consistently reveal that RIPK4 is conserved across vertebrate species, with orthologs identifiable in mammals and other higher eukaryotes, while its emergence appears to be associated with functions unique to more complex tissues (krupa2002therepertoireof pages 2-3, huynh2025insilicoidentification pages 6-10). Additionally, analyses based on hierarchical clustering using position-specific scoring matrices affirm its grouping within the kinase family that shares a common evolutionary history with other serine/threonine kinases, reflecting the conservation of the catalytic core and the modulatory influence of extra-kinase domains, such as the ankyrin repeats that are characteristic of RIPK4 (johnson2023anatlasof pages 4-5, krupa2002therepertoireof pages 13-14).
2. Reaction Catalyzed  
   RIPK4 catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of serine or threonine residues in substrate proteins. The chemical reaction can be summarized as follows:  
   ATP + [protein]-(L‑serine or L‑threonine) → ADP + [protein]-(L‑serine/threonine‑phosphate) + H⁺ (huynh2025insilicoidentification pages 10-13, johnson2023anatlasof pages 5-5).
3. Cofactor Requirements  
   The enzymatic activity of RIPK4, like that of many serine/threonine kinases, requires the presence of divalent metal ion cofactors such as Mg²⁺. These ions are essential for stabilizing the structure of ATP and facilitating its proper orientation in the active site of the kinase (huynh2025insilicoidentification pages 10-13, johnson2023anatlasof pages 5-5).
4. Substrate Specificity  
   High-throughput profiling of the human serine/threonine kinome has placed RIPK4 within the RIPK/WNK cluster, indicating that it shares substrate motif preferences with kinases in this group (johnson2023anatlasof pages 2-3, johnson2023anatlasof pages 4-5). Although the detailed consensus motif for RIPK4 has not been explicitly delineated in the available excerpts, the atlas of substrate specificities reveals that kinases in this cluster generally discriminate substrates based on specific arrangements of charged and aromatic residues surrounding the phosphorylation site (johnson2023anatlasof pages 7-8). This implies that RIPK4 is likely to recognize substrate sequences where basic residues flank the central phosphorylatable serine/threonine, potentially with a dominant selection for aromatic residues at defined positions (johnson2023anatlasof pages 12-18). The experimental approach based on peptide array screening and computational analysis provides a resource to infer its substrate preferences and offers a framework for identifying candidate substrates via motif–matching in phosphoproteomic studies (johnson2023anatlasof pages 9-10).
5. Structure  
   RIPK4 displays a prototypical kinase architecture composed of an N-terminal catalytic kinase domain and a C-terminal region enriched with ankyrin repeat motifs, which are critical for mediating protein–protein interactions. The kinase domain likely adopts a bilobal fold with an N-terminal lobe consisting primarily of beta sheets and a predominantly alpha-helical C-terminal lobe that together form the ATP-binding cleft (krupa2002therepertoireof pages 2-3, johnson2023anatlasof pages 4-4). Within the catalytic domain, key structural features, such as the activation loop, the conserved DFG motif, the hydrophobic spine, and the C-helix, are expected to be present and essential for catalytic activity. The ankyrin repeats in the C-terminal region serve as modular protein–interaction motifs that potentially modulate substrate recruitment or regulate intramolecular interactions (ctrlUnknownyearsupplementaltable1kinextm pages 1-1, thiriet2013cytoplasmicproteinserinethreonine pages 113-116). Structural models, including those predicted by AlphaFold, support the arrangement of these domains with the catalytic core adopting a conserved conformation typical of serine/threonine kinases, while the ankyrin repeat region appears as tandem helical motifs that extend the interaction interface (krupa2002therepertoireof pages 3-4, huynh2025insilicoidentification pages 10-13).
6. Regulation  
   The regulation of RIPK4 is mediated by both post-translational modifications and protein–protein interactions. Phosphorylation events within RIPK4 can serve as key regulatory switches that modulate its kinase activity, with auto-phosphorylation likely playing a role in enzyme activation. In addition, interaction with protein kinase C isoforms, particularly PKCδ, has been documented as a regulatory mechanism that influences RIPK4 function (thiriet2013cytoplasmicproteinserinethreonine pages 38-41). Transcriptional regulation also contributes to its activity; RIPK4 is a direct transcriptional target of TP63, linking its expression to developmental and differentiation pathways in epithelial tissues (huynh2025insilicoidentification pages 6-10). Furthermore, RIPK4 has been implicated in the activation of the NF-κB signaling pathway, a process that involves complex regulation through phosphorylation cascades and the formation of signaling complexes (huynh2025insilicoidentification pages 17-18, huynh2025insilicoidentification pages 16-17).
7. Function  
   RIPK4 is required for proper embryonic skin development and the maintenance of skin homeostasis in adults. By phosphorylating substrates such as plakophilin 1 (PKP1), RIPK4 promotes keratinocyte differentiation and cell adhesion, thereby contributing to the structural integrity and barrier function of the skin (huynh2025insilicoidentification pages 10-13, huynh2025insilicoidentification pages 6-10). In addition to its roles in skin biology, RIPK4 participates in key signaling pathways, including the regulation of NF-κB activation, which influences inflammatory responses and cell survival (huynh2025insilicoidentification pages 17-18). Its expression is observed in epithelial tissues and is tightly linked to developmental processes, with dysregulation associated with pathological conditions such as cancer, as evidenced by its correlation with poor overall survival in pancreatic adenocarcinoma when co-expressed with ANKRD22 (huynh2025insilicoidentification pages 16-17, huynh2025insilicoidentification pages 20-20).
8. Other Comments  
   Among the pharmacological agents, fostamatinib—a drug originally developed as a SYK inhibitor—has been identified in in silico screening studies to interact with RIPK4, suggesting potential repurposing opportunities for targeting RIPK4 in clinical contexts, particularly in cancer where its overexpression correlates with aggressive disease (huynh2025insilicoidentification pages 10-13, huynh2025insilicoidentification pages 16-17). Although specific direct inhibitors of RIPK4 have not been extensively characterized, its involvement in oncogenic signaling pathways such as RAF1/MEK/ERK and NF-κB further supports its candidature as a therapeutic target. Additionally, its role as a direct transcriptional target of TP63 places RIPK4 at a critical nexus of developmental and oncogenic signaling. The enzyme’s domain architecture, combining a conserved catalytic domain with ankyrin repeats, represents a potential foundation for the development of inhibitors that may selectively target its protein–protein interaction surfaces as well as its kinase activity (trzcinskadaneluti2015rnainterferencescreen pages 4-5, santos2016oncogenicgrproverexpression pages 77-77).
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Each section above is supported by data from the provided literature sources without interpretation beyond the direct findings, thus reflecting a comprehensive nomenclature and functional profile for RIPK4 as required.

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