1. Phylogeny  
   Ephrin type‑B receptor 2 (EPHB2) is a member of the Eph receptor family of receptor tyrosine kinases that is classified within the EphB subgroup. Members of this subgroup are conserved across vertebrate species and are present in diverse organisms ranging from early developmental model systems to mammals. Comparative genomic analyses indicate that orthologs of EPHB2 exist in a wide variety of species, consistent with the general evolutionary pattern that the Eph receptor family emerged from a common ancestral kinase that diversified during vertebrate evolution (fagotto2014ephrinephsignalingin pages 9-11). In the context of the human kinome, EPHB2 is grouped with other receptor tyrosine kinases whose evolution can be traced back to the early emergence of cell–cell communication mechanisms. This evolution is supported by analyses that compare domain architectures among Eph receptors; all members of the family share a conserved extracellular ligand-binding region and a cytoplasmic kinase domain, underscoring their shared origin. The conservation of these key domains throughout metazoan evolution reflects the importance of Eph receptor signaling in processes such as tissue patterning and cellular positioning (yaronbarir2024theintrinsicsubstrate pages 1-2). Phylogenetic studies have demonstrated that the EphB subgroup, including EPHB2, emerged via gene duplication events in early vertebrates, with subsequent specialization of ligand-binding affinity and intracellular regulatory mechanisms. This evolutionary pattern is typical of receptor tyrosine kinases that have diversified to fulfill multiple roles during embryogenesis and adult tissue homeostasis (fagotto2014ephrinephsignalingin pages 9-11, yaronbarir2024theintrinsicsubstrate pages 1-2).
2. Reaction Catalyzed  
   EPHB2 functions as a receptor tyrosine kinase; its catalytic activity consists of mediating the transfer of a phosphate group from ATP to specific tyrosine residues on substrate proteins. The general reaction catalyzed by EPHB2 can be represented as follows: ATP + [protein]-tyrosine → ADP + [protein]-phosphotyrosine + H⁺. This reaction not only includes the autophosphorylation of tyrosine residues on EPHB2 itself, which is critical for its activation, but also extends to phosphorylation of intracellular substrates that mediate downstream signaling events. The reaction mechanism utilizes ATP as a phosphate donor and results in the generation of ADP as a by‐product (yaronbarir2024theintrinsicsubstrate pages 17-19). This classical kinase reaction underlies the ability of EPHB2 to modulate cellular processes via phosphorylation‐dependent signaling cascades.
3. Cofactor Requirements  
   The kinase activity of EPHB2 is dependent on the presence of essential cofactors. In its catalytic reaction, ATP is required as the phosphate donor. In addition, typical for receptor tyrosine kinases, divalent cations—most notably Mg²⁺—are essential to stabilize the binding of ATP within the catalytic cleft of the kinase domain. The presence of Mg²⁺ facilitates the proper positioning of ATP and enhances the electrophilic character of the γ‑phosphate, thus promoting efficient phosphoryl transfer to the substrate tyrosine residue. These cofactor requirements are common among kinases and are indispensable for the enzymatic activity of EPHB2 (yaronbarir2024theintrinsicsubstrate pages 17-19).
4. Substrate Specificity  
   High‐throughput substrate profiling studies, including those employing comprehensive peptide arrays, have provided insights into the intrinsic substrate specificity of receptor tyrosine kinases such as EPHB2. The work by Yaron‑Barir et al. has revealed that the human tyrosine kinome exhibits distinct consensus motifs determined by the residues flanking the phosphorylated tyrosine (yaronbarir2024theintrinsicsubstrate pages 17-19). For EPHB2, experimental data indicate a substrate preference for peptide sequences containing acidic or polar residues immediately upstream of (–1 position) the central tyrosine. In addition, residues at the +3 position appear to be critical, with evidence suggesting an enriched selection for tryptophan or proline that likely contributes to favorable binding conformations and proper substrate orientation for phosphoryl transfer. This defined position‐specific amino acid preference enables precise recognition of phosphorylation sites and distinguishes EPHB2’s substrate specificity from that of other tyrosine kinases (yaronbarir2024theintrinsicsubstrate pages 17-19). Such a motif ensures that the downstream signaling cascades are activated only upon engagement with appropriate substrates, thus contributing to the fidelity of Eph receptor signaling in cellular contexts.
5. Structure  
   EPHB2 possesses the canonical architecture characteristic of Eph receptor tyrosine kinases. The extracellular region comprises an N‑terminal globular ligand‑binding domain that is responsible for interacting with transmembrane ephrin‑B ligands, followed by a cysteine‑rich domain and two fibronectin type‑III repeats that contribute to ligand binding and receptor clustering. This extracellular arrangement imparts the specificity and affinity required for direct cell–cell contact and bidirectional signaling. A single transmembrane helix anchors the receptor into the plasma membrane. The intracellular region of EPHB2 contains several distinct domains: a juxtamembrane segment that harbors autophosphorylation sites critical for relieving inhibitory conformations; a bilobal tyrosine kinase domain that is responsible for its catalytic activity; a sterile alpha motif (SAM) which is implicated in receptor oligomerization and protein–protein interactions; and a PDZ‑binding motif located at the extreme C‑terminus, which facilitates interactions with cytoskeletal and signaling adaptor proteins. Structural studies, supported by models and available crystallographic analyses of homologous Eph receptors, have shown that the tyrosine kinase domain of EPHB2 adopts a typical bilobal conformation with an N‑terminal lobe that contains a twisted five‑stranded beta sheet and a C‑terminal helical lobe featuring the activation loop, hydrophobic spine, and the critical C‑helix. The activation loop undergoes conformational changes upon autophosphorylation, thereby allowing substrate access to the catalytic site and stabilizing an active kinase conformation (fagotto2014ephrinephsignalingin pages 9-11). Such structural features are essential for the receptor’s function in signal transduction and underpin the regulation of its enzymatic activity.
6. Regulation  
   The regulation of EPHB2 is achieved via multiple interconnected mechanisms. Ligand binding by ephrin‑B family members induces receptor oligomerization and clustering, events that trigger conformational changes leading to autophosphorylation of critical tyrosine residues within the juxtamembrane region and kinase domain. This phosphorylation relieves autoinhibitory interactions, thereby activating the receptor’s catalytic function. Studies in neuronal systems have demonstrated that this ligand‑induced clustering is essential for both forward and reverse signaling, as it determines the extent and nature of intracellular signal propagation (strong2023activationofmultiplea pages 1-2, fagotto2014ephrinephsignalingin pages 16-17). In addition to ligand‑dependent activation, EPHB2 is regulated by dephosphorylation events mediated by protein tyrosine phosphatases that serve to attenuate the signal. Receptor internalization, via clathrin‑dependent endocytosis, further modulates signal duration by removing activated receptors from the cell surface. Proteolytic cleavage by metalloproteases, such as ADAM10, may also contribute to the regulation of EPHB2 by truncating the extracellular domain and dampening signal transduction (fagotto2014ephrinephsignalingin pages 17-18). Furthermore, modulatory interactions with intracellular adaptor proteins through the PDZ‑binding motif and SAM domain influence downstream signaling cascades, thereby integrating multiple layers of post‑translational regulatory control (strong2023activationofmultiplea pages 15-16).
7. Function  
   EPHB2 is implicated in a variety of biological processes through its role in mediating contact‑dependent bidirectional signaling. In developmental contexts, EPHB2 is essential for axon guidance; it directs the navigation of commissural axons, retinal ganglion cell axons, and contralateral inner ear efferents by translating ephrin‑B ligand interactions into repulsive and attractive signals that sculpt neural circuitry (fagotto2014ephrinephsignalingin pages 9-11, strong2023activationofmultiplea pages 1-2). In addition to its role in axon guidance, EPHB2 regulates dendritic spine development and maturation. Activation of EPHB2 modulates the intracellular network governing actin cytoskeletal dynamics, thereby stimulating the formation of excitatory synapses and contributing to the structural plasticity of neuronal networks. This capacity for modulating synaptic formation is critical in the establishment and maintenance of proper cortical connectivity and cognitive function (strong2023activationofmultiplea pages 1-2, fagotto2014ephrinephsignalingin pages 17-18). Beyond its functions in the nervous system, EPHB2 participates in organizing tissue boundaries and cell positioning during embryonic development. Through its interactions with ephrin‑B ligands on adjacent cells, EPHB2 coordinates cell adhesion and repulsion, thereby influencing spatial cell sorting and tissue morphogenesis. In many experimental systems, alterations in EPHB2 expression or function have been correlated with disruptions in tissue architecture and are linked to pathological conditions such as cancer, where loss or misregulation of Eph receptor signaling may contribute to tumor progression (fagotto2014ephrinephsignalingin pages 9-11, yaronbarir2024theintrinsicsubstrate pages 1-2).
8. Other Comments  
   EPHB2 is known by several alternative names, including Developmentally‑regulated Eph‑related tyrosine kinase, EPH tyrosine kinase 3, and Renal carcinoma antigen NY‑REN‑47, among others. Experimental modulation of EPHB2 signaling has been achieved using small molecule inhibitors such as NVP‑BHG712, which preferentially targets Eph receptor kinase activity, and this compound has been employed in studies of neuronal injury and synaptic remodeling (strong2023activationofmultiplea pages 15-16). Disease associations for EPHB2 are broad, with aberrant signaling implicated in neurodegenerative conditions, developmental disorders of the nervous system, and cancers including colorectal cancer. In the nervous system, altered EPHB2 signaling has been observed in models of optic neuropathy, where enhanced receptor phosphorylation correlates with synaptic destabilization and dendritic retraction. EPHB2 has also been linked to the regulation of excitatory synapse formation through its interaction with intracellular effectors that modulate transduction pathways involving Rho GTPases and actin dynamics. These findings underscore the therapeutic potential of targeting EPHB2 in disorders characterized by abnormal cell–cell communication and tissue architecture disruption (strong2023activationofmultiplea pages 15-16, fagotto2014ephrinephsignalingin pages 17-18). Its multifaceted role in regulating adhesion, repulsion, and synaptic organization positions EPHB2 as a critical modulator within the broader context of receptor tyrosine kinase signaling.
9. References  
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   [2] strong2023activationofmultiplea pages 1-2, pages 15-16  
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