## **Certificate of Analysis**



# ProQinase™ EPHB3

aa585-998

Product No.: 0231-0000-1

Lot: 001

**Description:** Human EPHB3 Amino acids Q<sub>585</sub>-V<sub>998</sub> (as in GenBank entry NM\_004443)\*, N-terminally fused to GST-HIS<sub>6</sub>-Thrombin cleavage site

\*Sequence may contain documented polymorphisms Detailed sequence on request

**Product identity:** EPHB3, Lot 001, was confirmed as human EPHB3 by mass spectroscopy LC-ESI-MS/MS (Protagen AG, Germany)

Theoretical MW<sub>Fusion Protein</sub>: 80,148 Da

Expression: Baculovirus infected Sf9 cells

**Purification:** One-step affinity purification using GSH-agarose

**Storage buffer:** 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

**Storage temperature:** -80°C Avoid repeated freeze-thaw cycles!

Protein concentration: 0.258 µg/µl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

## Method for determination of $K_m$ value & Specific activity:

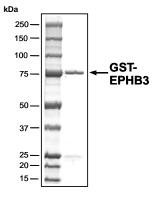
· Assay conditions:

60 mM HEPES-NaOH, pH 7.5 3 mM MgCl<sub>2</sub> 3 mM MnCl<sub>2</sub> 3  $\mu$ M Na-orthovanadate 1.2 mM DTT 2.5  $\mu$ g / 50  $\mu$ l PEG<sub>20.000</sub> ATP (variable) Substrate: Poly(Glu,Tyr)<sub>4:1</sub> (Sigma P-0275), 0.5  $\mu$ g / 50  $\mu$ l Recombinant EPHB3: 25 ng / 50  $\mu$ l

• Filter binding assay MAFC membrane (Millipore)

### Specific activity: 75 pmol/µg×min

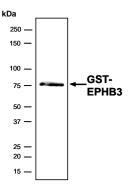
#### Coomassie stain:



2.0 µg GST-EPHB3

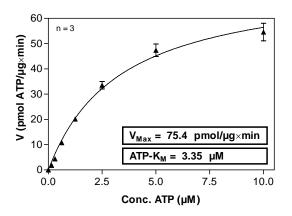
Western blot analysis:

anti-GST



0.5 µg GST-EPHB3

### Determination of K<sub>m</sub> value for ATP:



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