

## ProQinase™ EPHB4

aa563-987

**Product No.:** 0178-0000-3

**Lot:** 007

### Description:

Human EPHB4  
Amino acids L<sub>563</sub>-Y<sub>987</sub> (as in GenBank entry NM\_004444.2)\*, N-terminally fused to GST-HIS<sub>6</sub>-Thrombin cleavage site

\*Sequence may contain documented polymorphisms  
Detailed sequence on request

**Product identity:** EPHB4, Lot 007, was confirmed as human EPHB4 by mass spectroscopy LC-ESI-MS/MS (Protagen AG, Germany)

**Theoretical MW**<sub>Fusion Protein</sub>: 76,794 Da

**Expression:** Baculovirus infected Sf9 cells

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

**Activation:** pre-incubation with 0.1 mM ATP, followed by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation

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

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

**Protein concentration:** 0.235 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Method for determination of K<sub>m</sub> value & Specific activity:**

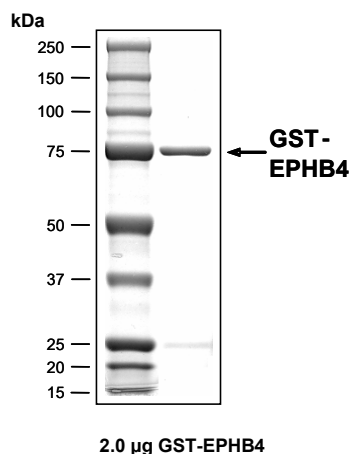
#### • Assay conditions:

60 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 µM Na-orthovanadate  
1.2 mM DTT  
2.5 µg / 50 µl PEG<sub>20,000</sub>  
ATP (variable)  
Substrate: Poly(Glu,Tyr)<sub>4:1</sub>  
(Sigma P-0275), 0.5 µg / 50 µl  
Recombinant EPHB4: 25 ng / 50 µl

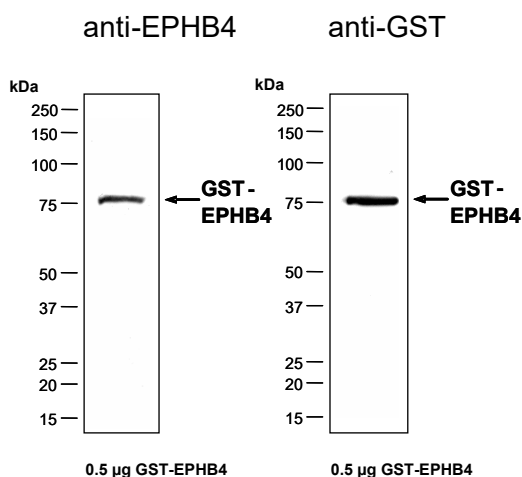
• Filter binding assay  
MAFC membrane (Millipore)

**Specific activity:** 78 pmol/µg×min

### Coomassie stain:



### Western blot analysis:



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

