

Certificate of Analysis

EPHA5 (EPH receptor A5)

Recombinant Human Active Protein Kinase

Synonyms: CEK7, EHK1, HEK7, TYRO4

Product No.: 0739-0000-1

Lot: 001

Description: Human EPHA5, C-terminal fragment, amino acids S₅₉₅-L₁₀₃₇ (as in GenBank entry NM_004439.3)*, activated, N-terminal GST-HIS₆ fusion protein with a Thrombin and a 3C cleavage site, expressed in Sf9 insect cells

*Sequence may contain documented polymorphisms
Detailed aa-sequence on request

Product identity: EPHA5, Lot 001, was confirmed as EPHA5 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,683 Da

Expression: Baculovirus infected Sf9 cells

Activation: in vitro auto-activation

Purification: GST-Affinity Chromatography

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

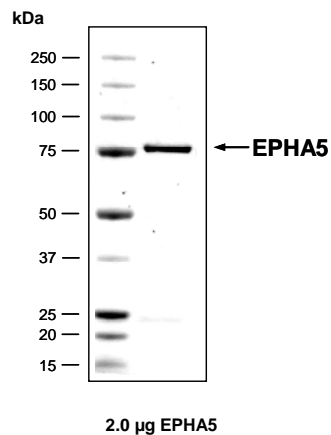
Protein concentration: 0.167 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Determination of K_m value & Specific activity:

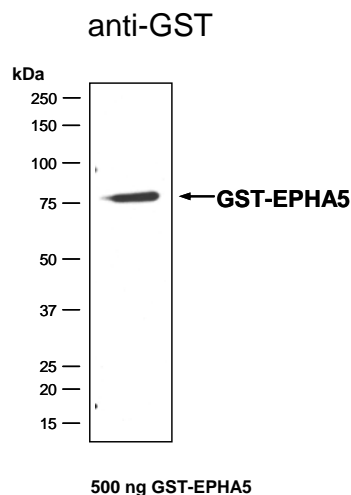
- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 2.5 µg / 50 µl PEG_{20,000}
 - ATP (variable)
 - Substrate: Poly(Glu,Tyr)_{4:1} (Sigma P-0275), 10 µg / ml
 - EPHA5: 400 ng / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Specific activity: 247 pmol/µg×min

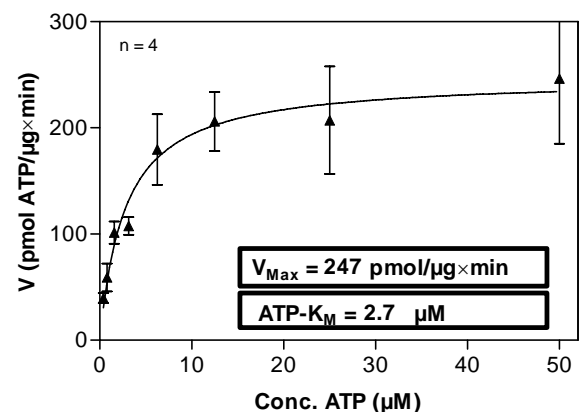
Coomassie stain:



Western blot analysis:



Determination of K_m value for ATP:



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