

Recombinant Proteins

ProQinaseTM 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),

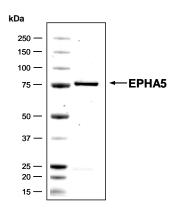
 $10 \, \mu g \, / \, ml$

EPHA5: 400 ng / ml
• Filter binding assay

MSFC membrane (Millipore)

Specific activity: 247 pmol/µg×min

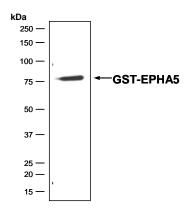
Coomassie stain:



2.0 µg EPHA5

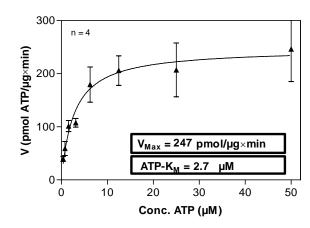
Western blot analysis:

anti-GST



500 ng GST-EPHA5

Determination of K_m value for ATP:



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