Certificate of Analysis

REACTION BIOLOGY

ProQinase™ EPHA1

aa569-976

Product No.: 0215-0000-1

Lot: 002

Description: Human EPHA1
Amino acids R_{569}-D_{976} (as in GenBank entry XM_056982)*, N-terminally fused to GST-HIS_6-Thrombin cleavage site

*Sequence may contain documented polymorphisms
Detailed sequence on request

Product identity: EPHA1, Lot 002, was confirmed as human EPHA1 by mass spectroscopy LC-ESI-MS/MS (Protagen AG, Germany)

Theoretical $MW_{Fusion\ Protein}$: 79,631 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: 0.071 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Method for determination of K_m value & Specific activity:

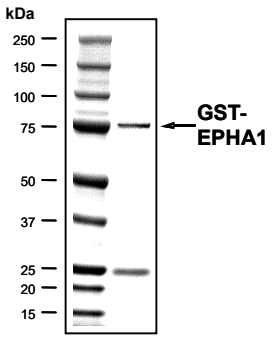
• Assay conditions:

60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
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), 2 µg / 50 µl
Recombinant EPHA1: 50 ng / 50 µl

• Filter binding assay
MAFC membrane (Millipore)

Specific activity: 4 pmol/µg×min

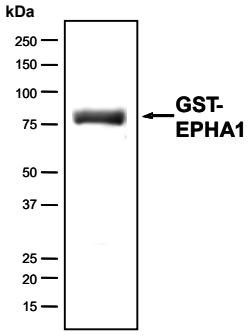
Coomassie stain:



2.0 µg GST-EPHA1

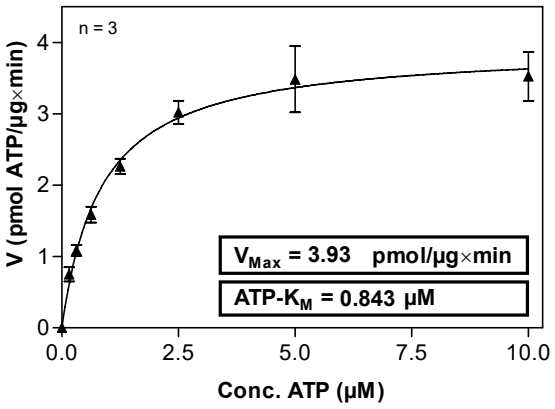
Western blot analysis:

anti-GST



0.5 µg GST-EPHA1

Determination of K_m value for ATP:



Recombinant Proteins