

AKT1

aa106-480

Product No.: 0132-0000-1

Lot: 007

Description: Human AKT1

Amino acids V₁₀₆-A₄₈₀ (as in GenBank entry NM_005163)*, N-terminally fused to GST-HIS₆-Thrombin cleavage site

*Sequence may contain documented polymorphisms
Detailed sequence on request

Product identity: AKT1, Lot 007, was confirmed as AKT1 by specific Western Blotting using anti AKT1 antibody

Theoretical MW_{Fusion Protein}: 77,832 Da

Expression: Baculovirus infected Sf9 cells

Activation: With PDK1
(GenBank accession No.: NM_002613)

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

Method for determination of K_m value & Specific activity:

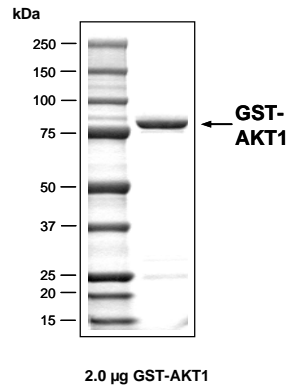
• Assay conditions:

- 60 mM HEPES-NaOH, pH 7.5
- 3 mM MgCl₂
- 3 mM MnCl₂
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- 2.5 µg / 50 µl PEG_{20,000}
- ATP (variable)
- Substrate: R₁₁-GSK3(14-27)
(R₁₁-SGRARTSSFAEPGGK), 5 µg / 50 µl
- Recombinant AKT1: 100 ng / 50 µl

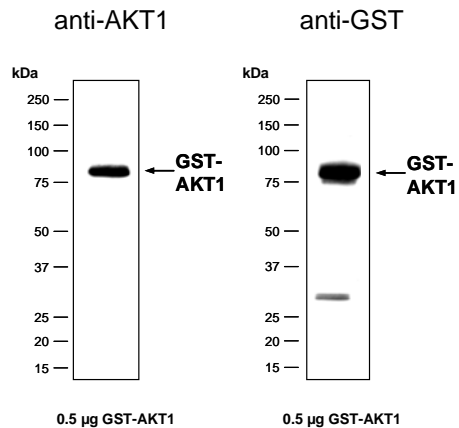
- Filter binding assay
MSPH membrane (Millipore)

Specific activity: 328 pmol/µg×min

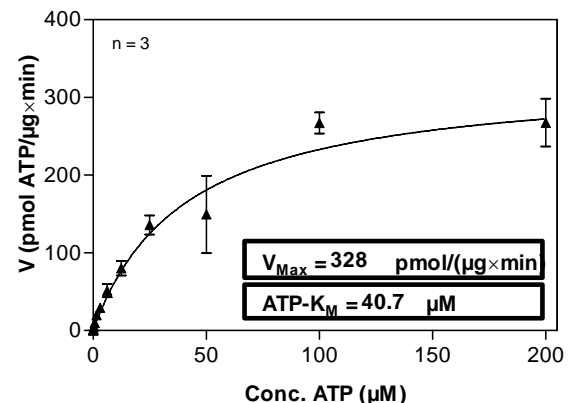
Coomassie stain:



Western blot analysis:



Determination of K_m value for ATP:



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