Store at -80°C

#7414

DDR2 Kinase

☑5 µg

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new 08/06

Description: Purified recombinant human DDR2 (Ser467-Glu855) kinase, supplied as a GST fusion protein.

Background: The discoidin domain receptors (DDRs) are receptor tyrosine kinases with a discoidin homology repeat in their extracellular domains, activated by binding to extracellular matrix collagens. So far, two mammalian DDRs have been identified: DDR1 and DDR2 (1). They are widely expressed in human tissues and may have roles in smooth muscle cell-mediated collagen remodeling (2). Aberrant expression and signaling of DDRs have been implicated in several human disease linked to accelerated matrix degradation and remodeling including tumor invasion, atherosclerosis and liver fibrosis (1).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing a fragment of human DDR2 (Ser467-Glu855) (GenBank Accession No. NM_006182) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

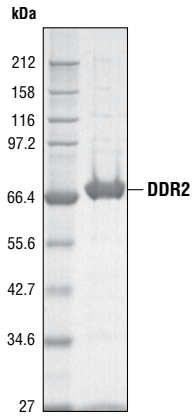

Figure 1. The purity of the GST-DDR2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-DDR2 fusion protein is 70 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. DDR2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Vogel, W. (1999) F*ASEB J.* 13 Suppl, S77–S82.

(2) Ferri, N. et al. (2004) *Am. J. Pathol.* 164, 1575–1585.

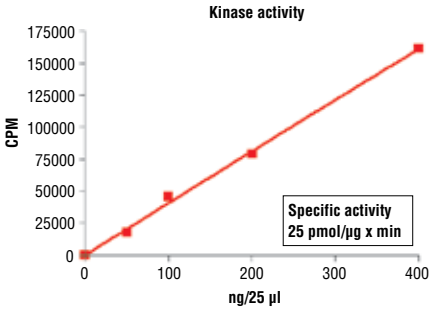

Figure 2. DDR2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 5 mM $MnCl_2$, 0.05 mM DTT, 50 ng/µl BSA, 50 µM ATP, Substrate: Axltide 400 ng/µL and recombinant DDR2: variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.
Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Protocol for DDR2 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (5X)**
   25 mM MOPS, pH 7.2
   2.5 mM β-glycerophosphate
   5 mM EGTA
   2 mM EDTA
   20 mM $MgC1_2$
   25 mM $MnCl_2$
   0.25 mM DTT
   250 ng/µl BSA
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Axltide (KKSRGDYMTMQIG) (1.0 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute DDR2 protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted DDR2 kinase solution, 10 µl Axltide (1.0 µg/µl) and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
0.4 mM EDTA
4 mM $MgCl_2$
5 mM $MnCl_2$
0.05 mM DTT
50 ng/µl BSA
400 ng/µL Axltide

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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