

DDR2, Active

Recombinant protein expressed in Sf9 cells

Catalog # 7760-5

Aliquot Size:	5 µg in 50 µl/vial
Concentration:	0.1 µg/µl
Purity:	>90%
Storage:	-80°C
Shipping:	in Dry ice
Shelf Life:	6-12 months from shipping date
Specific Activity:	25 nmol/min/mg

Product Description

Recombinant human DDR2 (467-end) was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM_006182](#).

Gene Aliases

TKT; NTRKR3; TYRO10

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

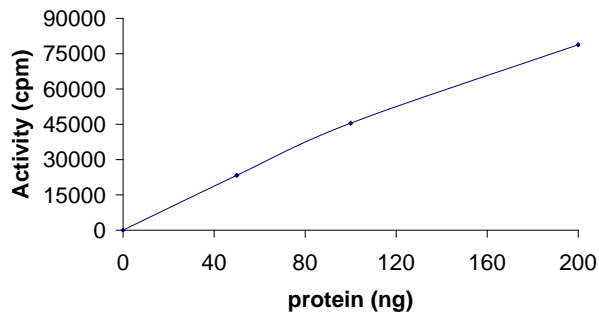
Scientific Background

DDR2 is a member of a novel subclass of RTKs containing a distinct extracellular region encompassing a factor VIII-like domain (1) and is thought to be involved in the regulation of cell growth, differentiation, and metabolism. DDR2 plays a role in the regulation of collagen turnover mediated by smooth muscle cells in obstructive diseases of blood vessels and the lung (2).

References

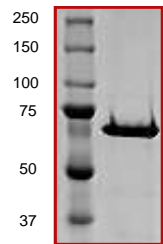
1. Kam, T. et al: Structure, expression and chromosomal mapping of TKT from man and mouse: a new subclass of receptor tyrosine kinases with a factor VIII-like domain. *Oncogene* 8: 3433-3440, 1993.
2. Ferri, N. et al: Role of discoidin domain receptors 1 and 2 in human smooth muscle cell-mediated collagen remodeling: potential implications in atherosclerosis and lymphangioleiomyomatosis. *Am J Pathol.* 2004 May;164(5):1575-85.

Specific Activity



The specific activity of DDR2 was determined to be **25 nmol / min / mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **70kDa**.

Activity Assay Protocol

Reaction Components

Active Kinase

Active DDR2 (0.1 µg/µl) diluted with Kinase Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active DDR2 for optimal results).

Kinase Dilution Buffer, pH 7.2

Kinase Assay Buffer II diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer II, pH 7.2

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 25mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution, 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer. Store 1ml aliquots at -20°C.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200µl aliquots at -20°C.

Substrate

Axltide synthetic peptide substrate (KKS^RGDYMTMQIG) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active DDR2, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active DDR2.
 - Component 2.** 10µl of 1mg/ml stock solution of substrate
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

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- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³²P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]