

## Product: PYK2, Active

Catalog #: 02-2080

Amount: 5 µg

### Product Description

Recombinant human PYK2 (360-690) was expressed in Sf9 cells using an N-terminal His tag. The gene accession number is [NM\\_004103](#).

### Gene Aliases

FAK2, PTK2B, CADTK, FADK2, CAKB, PKB, PTK, RAFTK

### 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.

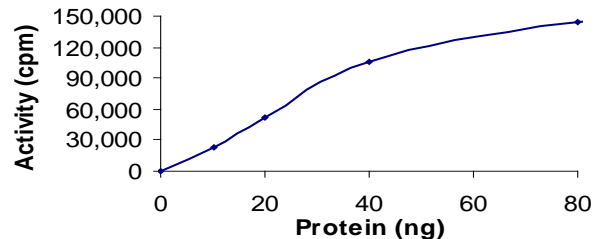
### Scientific Background

PYK2 (also known as FAK2/RAFTK) is a member of the focal adhesion PTK family. PYK2/FAK2 can be activated by a variety of extracellular signals that elevate intracellular calcium concentration, and by stress signals (1). Unlike FAK, which is widely expressed in various tissues and links transmembrane integrin receptors to intracellular pathways, PYK2/FAK2 is expressed mainly in the central nervous system and in cells derived from hematopoietic lineages. In osteoclasts, although FAK is expressed, PYK2/FAK2 appears to be the predominant mediator of integrin alpha(v)beta3 signaling events that influence osteoclast physiology and pathology (2).

### References

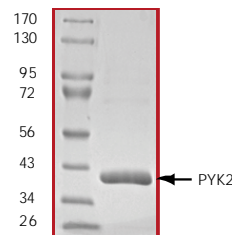
1. Avraham, H. et al: RAFTK/Pyk2-mediated cellular signalling. Cell Signal. 2000 Mar;12(3):123-33.
2. Xiong, W C. et al: PYK2 and FAK in osteoclasts. Front Biosci. 2003 Sep 1:8:d1219-26.

### Specific Activity



The specific activity of PYK2 was determined to be **254 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **~39kDa**.

## PYK2, Active

Human recombinant protein expressed in Sf9 cells

Catalog Number	<b>02-2080</b>
Quantity	5µg
Specific Activity	254 nmol/min/mg
Specific Lot Number	P022-1
Purity	>90%
Format	5µg in 50µl
Concentration	0.1 µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

## Activity Assay Protocol

### Reaction Components

#### Active Kinase (Catalog #: 02-2080)

Active PYK2 (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K24-09) 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 PYK2 for optimal results).

#### Kinase Dilution Buffer, pH 7.2 (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 µg/ml BSA solution.

#### Kinase Assay Buffer II, pH 7.2 (Catalog #: K02-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 20mM , 12.5mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

#### [<sup>32</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>32</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K02-09). Store 1ml aliquots at -20°C.

#### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K02-09). Store 200µl aliquots at -20°C.

#### Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled to a final concentration of 1 mg/ml.

### Assay Protocol

- Step 1. Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PYK2, 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 PYK2 (Catalog # 02-2080)
  - Component 2. 10µl of 1 mg/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 H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl 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 H<sub>2</sub>O) 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 [<sup>32</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

#### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.