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| Catalog # | Aliquot Size |
|---|---|
| P13-19DG-05 | 5 µg |
| P13-19DG-10 | 10 µg |

# TPM3-PDGFR beta, Active

Recombinant human fusion protein expressed in Sf9 cells

**Catalog # P13-19DG**

*Lot # K1730-1*

## Product Description

Recombinant human TPM3-PDGFRB, the fusion protein [TPM3 (1-258)-PDGFRB (528-end)], was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TPM3 gene accession number is NM_152263 and PDGFRB's one is NM_002609.

## Gene Aliases

TPM3: CAPM1; CFTD; hscp30; NEM1; OK/SW-cl.5; TM-5; TM3; TM30; TM30nm; TM5; TPMsk3
PDGFRB: JTK12, PDGFR, CD140B, PDGFR1, PDGF-R-beta

## Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

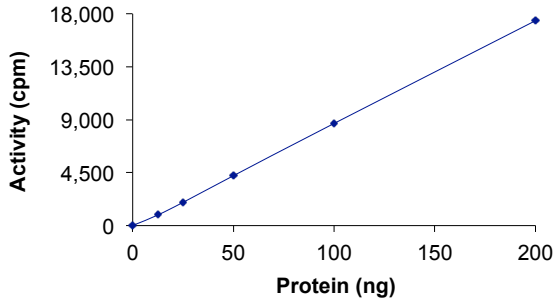
## Scientific Background

TPM3 is an actin-binding protein that mediates myosin–actin response to calcium ions in skeletal muscles. PDGFRB is a receptor tyrosine kinase of the PDGFR family that binds members of the platelet-derived growth factor family. PDGFRB is located at chromosome 5q31-33, and has been reported to have at least 18 fusion partners. It was uncovered that the TPM3 gene at 1q21 as a PDGFRB partner in chronic eosinophilic leukemia (CEL).

## References

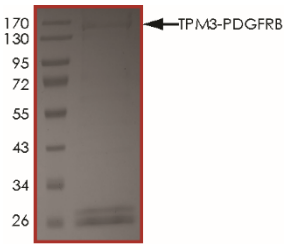

1. Rosati R, et al. TPM3/PDGFRB fusion transcript and its reciprocal in chronic eosinophilic leukemia. Leukemia. 20(9):1623-4, 2006.
2. Li Z, Yang R, Zhao J, Yuan R, Lu Q, Li Q, Tse W. Molecular diagnosis andtargeted therapy of a pediatric chronic eosinophilic leukemia patient carrying TPM3-PDGFRB fusion. Pediatr Blood Cancer. 56(3):463-6. 2011.


## Specific Activity




The specific activity of TPM3-PDGFRB was determined to be **4.6 nmol/min/mg** as per activity assay protocol.

## Purity




The purity of TPM3-PDGFRB was determined to be **>70%** by densitometry, approx. MW **150 kDa**.


# TPM3-PDGFR beta, Active

Recombinant human fusion protein expressed in Sf9 cells


| | |
|---|---|
| Catalog # | P13-19DG |
| Specific Activity | 4.6 nmol/min/mg |
| Lot # | K1730-1 |
| Purity | >70% |
| Concentration | 0.05 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. |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# Activity Assay Protocol

## Reaction Components

**Active Kinase** (Catalog #: P13-19DG)

Active TPM3-PDGFRB (0.05μg/μl) diluted with Kinase Dilution Buffer IV (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 TPM3-PDGFRB for optimal results).

**Kinase Dilution Buffer IV** (Catalog #: K24-09)

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

**Kinase Assay Buffer II** (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 25mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[$^{33}$P]-ATP Assay Cocktail**

Prepare 250μM [$^{33}$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 [$^{33}$P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (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 II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

**Substrate** (Catalog #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIEFG) diluted in distilled $H_2O$ to a final concentration of 1mg/ml.

## Assay Protocol

**Step 1.** Thaw [$^{33}$P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

**Step 2.** Thaw the Active TPM3-PDGFRB, Kinase Assay Buffer, Substrate and Kinase 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 TPM3-PDGFRB (Catalog #P13-19DG)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog # I15-58)
- **Component 3.** 5μl distilled $H_2O$ (4°C)

**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 $H_2O$.

**Step 5.** Initiate the reaction by the addition of 5 μl [$^{33}$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 20 μl 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 $H_2O$) 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 [P$^{33}$]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [$^{33}$P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of $^{33}$P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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