

## MET, Active

Recombinant protein expressed in Sf9 cells

Catalog # 7771-5

Lot # \_\_\_\_\_

## Product Description

Recombinant rat MET (958-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [X96786](#).

## Gene Aliases

HGFR

## 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  $-70^{\circ}\text{C}$ . 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.

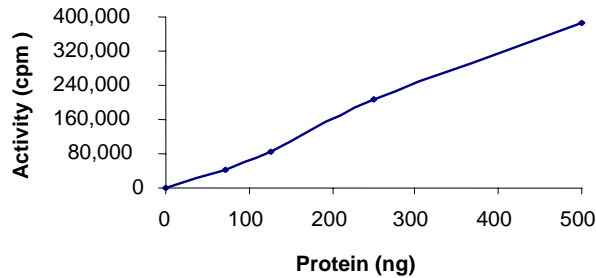
## Scientific Background

MET is a proto-oncogene that encodes a transmembrane growth factor receptor which is a heterodimer of two disulphide linked chains of 50 kd (alpha) and 145 kd (beta). MET is widely expressed in the kidney, brain, lung, skin, and embryonic tissue (1). Hepatocyte growth factor (HGF) binds to MET and activates its tyrosine kinase activity. MET is overexpressed and activated in a variety of human cancers including pancreatic, colon, gastric, cervical and ovarian cancers and has been shown to be involved in tumor cell migration and invasion (2).

## References

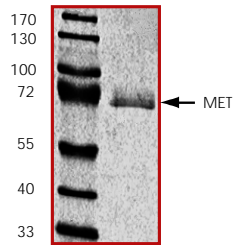
1. Giordano, S. et al: Biosynthesis of the protein encoded by the c-met proto-oncogene. *Oncogene*. 1989 Nov;4(11):1383-8.
2. Iyer, A. et al: Structure, tissue-specific expression, and transforming activity of the mouse met protooncogene. *Cell Growth Differ*. 1990 Feb;1(2):87-95.

## Specific Activity



The specific activity of MET was determined to be **46 nmol /min/mg** as per activity assay protocol.

## Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **74kDa**.

# Activity Assay Protocol

## Reaction Components

### Active Kinase

Active MET (0.1 $\mu$ g/ $\mu$ l) 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 MET for optimal results).

### Kinase Dilution Buffer, pH 7.2

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

### Kinase Assay Buffer II, pH 7.2

Buffer components: 25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 20mM MgCl<sub>2</sub>, 25mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

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

Prepare 250 $\mu$ M [<sup>32</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution, 100 $\mu$ l [<sup>32</sup>P]-ATP (1mCi/100 $\mu$ l), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). 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 200 $\mu$ l aliquots at -20°C.

### Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H<sub>2</sub>O 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 MET, 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 20 $\mu$ l:
- Component 1.** 10 $\mu$ l of diluted Active MET.
- Component 2.** 10 $\mu$ l 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 H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 $\mu$ l [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 $\mu$ 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 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 5 $\mu$ l [<sup>32</sup>P]-ATP / pmoles of ATP (in 5 $\mu$ l of a 250 $\mu$ M ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/ $\mu$ g 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  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]