Met. Sf9 cells Active Enzyme

Background

When HGF/SF activates c-Met, the first proteins to be activated downstream are Grb2 (growth factor receptor bound protein 2) and Gab 1 (growth factor receptor bound protein 2 associated binder 1). Grb2 in turn may activate a number of kinase pathways, including the pathway from Ras to Raf to Mek and to MAPK(mitogen-activated protein kinase). Gab 1 activates PI3K (phosphoinositide 3 kinase), which activates STAT3 (signal transducer and activator of transcription). c-Met activation also induces activation of beta catenin, a key component of the wnt pathway, which translocates into the nucleus and participates in transcription regulation.

The HGF/c-Met pathway plays an important role in the development of cancer. First through the activation of key oncogenic pathways (Ras, PI3K/STAT3, beta catenin), secondly through endothelial cell proliferation (neoangiogenesis), thirdly through increased protease production and hence cell dissociation leading to metastasis.

Ordering Information

Catalog Number
X1761E

Size
10 μg

Customer Storage
Product should be stored at -80ºC. Aliquot to avoid freeze/thaw cycles

Formulation
Provided in 50mM Tris (pH 7.5) solution containing 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol

Ship Conditions
Ship on dry ice, freeze upon arrival

Stability
Products are stable for one year from purchase when stored properly

Concentration
Lot specific, see vial

Source
Baculovirus infected Sf9 cells

Activity

46 units/μg. One unit is defined as the amount of enzyme that will transfer 1 pmol phosphate to Tyr substrate per minute at pH 7.4 and 30ºC. Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Purity

>65% by densitometry

Applications

Enzyme Kinetic studies, screening inhibitors, selectivity profiling

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Assay Methods

Materials

Assay Buffer (5X): 100 mM HEPES, pH 7.5, 50 mM $MgCl^2$, 5 mM $MnCl^2$, 5 mM DTT, 5 mM EDTA.
Sodium orthovanadate (50 mM) solution)
Substrate: 1 μg/ml biotin-conjugated poly-(Glu_4-Tyr) substrate
ELISA Blocking Buffer (1% BSA in TBST)
ELISA Plate Wash Buffer (TBST)
Phosporylated Tyrosine(PY)-specific antibody conjugated to HRP: (Cat. No. X1018)
TMB substrate solution
TMB Stop Solution
ELISA Plates
Plate reader capable of reading 450 nm

Procedure

1. Add 100μl of ELISA Blocking Buffer to streptavidin-coated microwell plate, incubate at RT for 15 min.
2. Remove block; add 100μl of Substrate solution (1μg/ml). Incubate for 1 hour at 37ºC.
3. Wash plate 3 times with 1X TBST
4. Add 300 μl of Block and incubate at 37ºC for 1-2 hours or overnight at 4ºC
5. Set up reactions: Water (to 50μl), 5X Assay Buffer (10μl), 5X ATP Solution (10μl), Sodium orthovanadate (50 mM0 (1μl), PTK enzyme (100 ng, 500 ng).
6. Incubate at 37ºC for 30 min.
7. Wash 4 times with wash buffer.
8. Add 200 μl of block to each well and incubate at RT for 15 min.
9. add 100 μl of anti-PY-HRP (1:500 in block to each well and incubate for 30 min.
10. wash 4 times with 1x wash buffer.
11. Add 100 μl of TMB to each well and incubate 5 -15 min.
12. Add 100 μl of stop solution to each well.
13. Read absorbance at 450 nm.

References

1. Giordano, S. et al., Oncogene, 1989, 4: 1383-1388.

2. Lyer, A. et al., Cell Growth Differ. 1990, 1: 87-95.

3. Emaduddin M. et al., Proc. Natl. Acad. Sci. U.S.A. 105 (7):2358-2362 (2008).

4. Drebber U. et al., Oncol Rep. 19(6):1477-83 (2008).

Product Specific References