



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.

ACTIVITY

46 units/ μ g. 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 30°C. Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

PURITY

>65% by densitometry

APPLICATIONS

ORDERING INFORMATION

CATALOG NUMBER

X1761E

SIZE

10 μ g

CUSTOMER STORAGE

Product should be stored at -80°C.
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

Stable for > 6 months when stored as recommended

CONCENTRATION

Lot specific, see vial

SOURCE

Baculovirus infected Sf9 cells

ASSAY METHODS

MATERIALS

Assay Buffer (5X): 100 mM HEPES, pH 7.5, 50 mM MgCl₂, 5 mM MnCl₂, 5 mM DTT, 5 mM EDTA.
Sodium orthovanadate (50 mM) solution)
Substrate: 1 µg/ml biotin-conjugated poly-(Glu₄-Tyr) substrate
ELISA Blocking Buffer (1% BSA in TBST)
ELISA Plate Wash Buffer (TBST)
Phosphorylated 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 37°C.
3. Wash plate 3 times with 1X TBST
4. Add 300 µl of Block and incubate at 37°C for 1-2 hours or overnight at 4°C
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 37°C 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

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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

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