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ProductInformation

MAP KINASE ACTIVATED
Product Number **M 3172**

Product Description
Rat, Recombinant, N-terminal Histidine tagged protein, Expressed in *E. coli*., 42 kDa

Solution containing 20 mM Tris buffer pH 7.5, 150 mM NaCl, 1 mM EGTA, 1 mM DTT, 10% glycerol and 0.03%Brij

MAP kinase is a serine/threonine kinase that is activated by phosphorylation in a cascade of events initiated by extracellular signals. Such signals are transmitted via growth factor regulated receptor tyrosine kinase or modulated through a G protein coupled receptor. The threonine and tyrosine dual phosphorylated MAP kinase is translocated from the cytoplasm to the nucleus. In the nucleus, MAP kinase phosphorylates proteins, such as transcription factors, and relays the extracellular signal into a genomic response leading to proliferation or differentiation.

Storage/Stability
Store at –70 °C.

Procedure
Assay: The activity of MAP Kinase activated is determined by phosphorylation of Myelin Basic protein (MBP).

Assay principle: MBP is phosphorylated using [32P-γ] ATP. The phosphorylated protein is separated from the reactive mixture by absorption on Whatman p-81 phosphocellulose paper. After extensive washings with 0.5% phosphoric acid, the radioactivity absorbed to the paper is measured.

Reagents:
- Enzyme dilution buffer (EDB) Glycerophosphate pH 7.3, 3 mM DTT, 0.1 mM Na Vanadate, 1.5 mM EGTA, 1 mM EDTA. Keep the solution at –20 °C
- Activated MAP Kinase (ERK2), dilute to 20µg/ml using EDB
- MBP (M-1891) 6 mg/ml in water)
- [32P-γ] ATP 3000 Ci/mmol, 1 mCi
- Reaction buffer x 3 (RBX3): 75 mM β-Glycerophosphate pH 7.3, 30 mM $MgCl_2$, 4.5 mM DTT, 0.15 mM Na Vanadate, 3.75 mM EGTA, 300 µM ATP, add 1 µl [32P-γ] ATP of 10 mCi per 1ml to 100 µl RBX3 before use.
- 0.5% phosphoric acid in DDW (18 ml phosphoric acid 85% in 3 L water)
- Whatman p-81 phosphocellulose paper, cut into 1.5 x 5 cm pieces, the tube number marked on each pieces with pencil.
- Ethanol
- Acetone

Procedure
The assay is performed in duplicate

Reaction scheme

	ERK2	EDB	MBP	RBX3
Blank	------	10 µl	10 µl	10 µl
Sample	10 µl	------	10 µl	10 µl
Incubate at 30 °C for 3 minutes				

1. Dilute MAP Kinase Activated to 20 µg/ml with EDB.
2. Pipette 10 µl of diluted MAP Kinase Activated into an eppendorf tube.
3. Pipette 10 µl of EDB for blank into a second eppendorf tube.
4. Add 10 µl of MBP solution to the tubes.
5. Start the reaction by adding 10 µl of RBX3.
6. Vortex gently for a few seconds, and incubate for 3 min. at 30 °C.
7. Apply 20 µl aliquot of each reaction mixture to a piece of phosphocellulose P-81 (1.5 x 5 cm). Soak the pieces in 0.5% phosphoric acid.
8. Wash the pieces of paper 4 times with 0.5% phosphoric acid. Agitate gently each wash for 5-6 min.
9. Wash once with ethanol for 1 min.
10. Wash once with acetone for 1 min.
11. Dry the pieces of paper at room temperature or under a heat lamp and measure isotopic incorporation using Cerenkov mode (i.e. count the β- emission without scintillation liquid, using tritium channel).

Calculation

1. Count **R**, the radioactivity of 10 µl of RBX3, in order to obtain the total radioactivity in cpm per assay tube (perform in duplicate).
2. Divide the above value **R** by the amount of ATP present in the assay tube (3 nmole) in order to obtain the specific radioactivity, **SR= R/3.0** (cpm/nmol)
3. Count the sample and multiply the result by a factor 30/20, C = count of sample X30/20
4. Calculate the amount of MAP kinase protein in the assay= mgP
5. $\frac{C}{SR \times 3 \times mgP}$ = U, nmol/min/mg

Product Profile

Purity: >95% (SDS-PAGE)

Activity: More than 500 U/mg

Unit definition: 1 unit of activated MAP kinase (ERK2) will transfer 1 nmole of phosphate from ATP to myelin basic protein per 1 min at 30 °C.

References

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