Certificate of Analysis

MEK Inhibitor U0126:

Part# 9PIV112

Revised 8/16

Part No.	Size
V112A	5 × 1mg

Description: MEK Inhibitor U0126 is a chemically synthesized organic compound that inhibits the kinase activity of MAP Kinase Kinase (MAPKK or MEK). It has been used in both in vivo and in vitro studies of MEK (1–6).

Formula Weight: 426.5 Daltons.

Reconstitution: MEK Inhibitor U0126 is supplied as dried material. Resuspend 1mg of U0126 (one vial) in 234µl of DMSO and vortex vigorously for 30 seconds to produce a stock solution of 10mM. Further dilutions can be carried out in DMSO. Once resuspended in DMSO, store at –20°C. The resuspended product is stable for no more than one week (6).

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

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Quality Control Assays

Identity: The chemical identity and the isomeric purity of MEK Inhibitor U0126 is confirmed by proton NMR spectroscopy. Purity is checked by elemental analysis.

Promega

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References

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Signed by:

R. Wheeler, Quality Assurance

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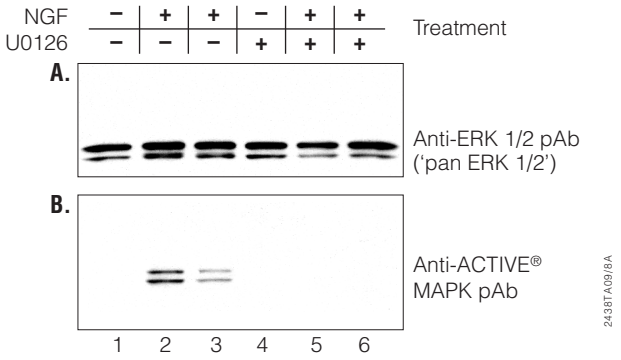
Usage Information

1. Example Protocol

The protocol given below has been used successfully at Promega to demonstrate the inhibitory effect of MEK Inhibitor U0126 on ERK1/2 signaling cascade in the presence of NGF (1). However, this procedure is not performed as a quality control assay on each batch of the MEK Inhibitor U0126 and will require optimization depending on the experimental conditions used.

Inhibitory effect of U0126 on ERK1/2 signaling cascade in the presence of NGF.



PC12 cells were grown in serum-free RPMI medium for 16 hours. The inhibitor U0126 was added to the medium at 10µM in 0.2% DMSO for 15 minutes. Nerve growth factor (NGF) was then added at a final concentration of 50ng/ml, and the cells were incubated for either 5 or 30 minutes before harvesting and lysing. Protein samples (20µg) were analyzed by SDS-PAGE and Western blot. Detection was performed with Anti-ERK 1/2 pAb (Cat.# V1141) and Anti-ACTIVE® MAPK pAb (Cat.# V8031) using the Tropix chemiluminescence detection system. **Panel A.** The amount of total ERK1/2 shows little change with the various treatments, as detected by the Anti-ERK 1/2 pAb. **Panel B.** Using Anti-ACTIVE® MAPK pAb, which recognizes only the active ERK1/2, it is shown that U0126 completely inhibits the activation of ERK1/2 in NGF-treated PC12 cells at both 5 and 30 minutes. Lane 1, no NGF; lane 2, NGF for 5 minutes; lane 3, NGF for 30 minutes; lane 4, U0126; lane 5, U0126 + NGF for 5 minutes; lane 6, U0126 + NGF for 30 minutes.

2. Related Products

Product	Size	Cat.#
Anti-ACTIVE® MAPK pAb, Rabbit (pTEpY)	40µl	V8031
Anti-ACTIVE® JNK pAb, Rabbit (pTPpY)	40µl	V7931
	120µl	V7932
Anti-ACTIVE® p38 pAb, Rabbit (pTGpY)	100µl	V1211
Anti-ERK 1/2 pAb, Rabbit	40µl	V1141
Donkey Anti-Rabbit IgG, HRP, Anti-ACTIVE® Qualified	60µl	V7951
SB 203580 (p38 Inhibitor)	1mg	V1161
PD 98059 (MEK Inhibitor)	5mg	V1191

3. Reference

1. Goueli, S.A. *et al.* U0126: A novel, selective and potent inhibitor of MAP Kinase (MEK). *Promega Notes* **69**, 6–8.

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