



Certificate of Analysis

MAP Kinase Assay Kit (Non-Radioactive) Catalog # 17-191 Lot # DAM1547748

Kit Components

Assay Dilution Buffer I (ADBI), Catalog # 20-108. Two vials, each containing **1ml** of ADBI: 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

Anti-phospho-MBP, clone P12, Catalog # 05-429. One vial containing **100 μ g** of protein A purified IgG_{2a} in **100 μ l** of PBS, pH 7.4, 0.09% sodium azide before the addition of glycerol to 30%.

Magnesium/ATP Cocktail, Catalog # 20-113. Two vials, each containing **1ml** of Mg²⁺/ATP cocktail: 75mM magnesium chloride and 500 μ M ATP in ADBI.

MAP Kinase Substrate Cocktail II, Catalog # 20-166. Two vials, each vial containing **1ml** of substrate cocktail: 2mg/ml dephosphorylated myelin basic protein in ADBI.

Inhibitor Cocktail, Catalog # 20-116. One vial containing **1ml** of inhibitor cocktail: 20 μ M PKC inhibitor peptide (Catalog # 12-121) 2 μ M PKA inhibitor peptide (PKI) (Catalog # 12-151), and 20 μ M Compound R24571 in ADBI.

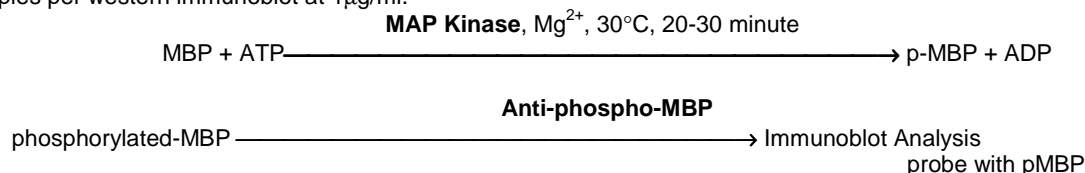
FOR IN VITRO RESEARCH USE ONLY
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS
DO NOT USE IN HUMANS OR IN ANIMALS

Kit Description

Quantity: 100 kinase assays per kit, if analyzed as 10 samples per western immunoblot at 1 μ g/ml.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the components, centrifuge the original vial after thawing and prior to removing the cap.

Use: The assay kit is designed to measure the phosphotransferase activity of MAP (mitogen-activated protein) Kinase in immunoprecipitates and column fractions. Crude cell lysates may also be used but detergents/biochemicals contained in the cell lysis buffer may inhibit MAP Kinase activity. Furthermore, although three inhibitors are included with the kit, editors may suggest other unknown kinases found in crude lysates are responsible for MBP phosphorylation. The assay kit is based on phosphorylation of a specific substrate (myelin basic protein, MBP). The phosphorylated substrate is then analyzed by immunoblot analysis, probing with a monoclonal Phospho-specific MBP antibody. The enzyme assay is rapid, convenient and specific for MAP Kinase and contains sufficient reagents for 100 individual MAP Kinase assays, if analyzed as 10 samples per western immunoblot at 1 μ g/ml.



Other components required but not included as part of kit are:

- A preparation containing MAP Kinase, such as 10-200µg of MAP Kinase immunoprecipitate diluted into ADBI or 25-100ng of purified MAP Kinase.
- TBS
- 2X Laemmli sample buffer
- vortex mixer
- 30°C shaking incubator
- timer
- variable volume (5-200µl) pipet + tips
- Immunoblot reagents and materials

Safety Warnings and Precautions: The Non-radioactive MAP Kinase assay kit is designed for research only and not recommended for internal use in humans or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.

MAP Kinase Assay Kit Overview

All assay components must be rapidly thawed and mixed completely before proceeding with assay. Do not use extended thawing time. Once the assay components are thawed, mix thoroughly by vortexing. The assay components can be refrozen at -20°C for extended periods.

Perform all preincubation reactions at 1°C over an ice bath. The kinase assay can also be performed at room temperature but will not give linear results. Aliquot 10µl of substrate cocktail, 10µl of inhibitor cocktail and 10µl of enzyme preparation into the bottom of a microcentrifuge tube. Start the reaction by adding 10µl of the Mg²⁺/ATP cocktail, vortex gently and incubate the microcentrifuge tube shaking at 30°C for 20-30 minutes. Remove and dilute an aliquot so that approximately 0.5-1µg of phosphorylated MBP is analyzed by immunoblot analysis.

Suitable blanks should always be performed to correct for non-specific binding. Controls for endogenous phosphorylation of proteins in the sample extract can be performed by substituting ADBI for substrate cocktail. Controls for the basal level of phosphorylated MBP should be run to compare the background basal level (noise) of the MBP substrate and the phosphorylated MBP signal on the immunoblot.

Assay Protocol:

1. Add 10µl of Mg²⁺/ATP cocktail to a microcentrifuge tube.
2. Add 10µl of ADBI.
3. Add 10µl (20µg) of Map Kinase substrate cocktail.
4. Add 10µl of the inhibitor cocktail (optional control) or ADBI.
5. Add 10µl of an active MAP Kinase preparation (25-100ng purified enzyme/assay or 10-200µg immunoprecipitated protein).
6. Incubate for 20-30 minutes in a 30°C shaking incubator.

Note: Components must be thoroughly mixed throughout the reaction time to ensure that the MBP, and the enzyme achieve maximum interaction.

7. Remove 2.5µl of the reaction mixture (approximately 1µg of phosphorylated-MBP) and place into another centrifuge tube. Add 7.5µl of TBS and 10µl of 2X Laemmli sample buffer. Load an aliquot of the sample for SDS-PAGE and immunoblot analysis.

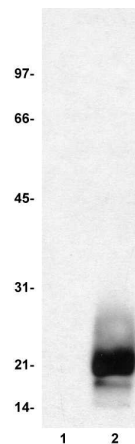
Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on the experimental samples and transfer the phosphorylated MBP to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-phospho-MBP, clone P12**, diluted in freshly prepared TBS-MILK overnight with agitation at 4 $^{\circ}$ C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-mouse** HRP conjugated IgG, catalog # 12-349, 1:2000 dilution was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-0.05% Tween-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Quality Control Testing

Immunoblot Analysis:

Representative blot from a previous lot. MAP Kinase was used to phosphorylate myelin basic protein (MBP) *in vitro*. The results of an immunoblot analysis from an *in vitro* assay are shown to the right. Lane 1: basal level of MBP (1 μ g) phosphorylation; Lane 2: MBP incubated with MAP Kinase 2/Erk2, active (Catalog # 14-173). The immunoblot was probed using 1 μ g/ml anti-phospho-MBP, clone P12.



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28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 800 437-7500 • F: 800 437-7502
www.millipore.com