



cell signaling solutions

Certificate of Analysis

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p38α/SAPK2a Assay Kit

Catalog # 17-169

Lot # 27203

Kit Components

p38α/SAPK2a, active, Catalog # 14-210, Lot # 26512; see page two for more information. One vial containing 5µg recombinant protein in 50µl of 50mM Tris-HCl, pH 7.5, 270mM sucrose, 150mM NaCl, 0.1mM EGTA, 1mM benzamidine, 0.03% Brij-35, 0.1% β-mercaptoethanol, 0.2mM PMSF.

MAPKAP Kinase 2, unactive, Catalog # 14-216, Lot # 23658; see page two for more information. One vial containing 10µg of recombinant protein in 50µl of 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 0.1% (v/v) 2-mercaptoethanol, 1mM benzamidine, 0.2mM PMSF and 50% glycerol.

P81 Phosphocellulose Squares, Catalog # 20-134. One pouch containing 200 prelabeled squares.

MAPKAP Kinase 2 Substrate Peptide: Catalog # 12-240, Lot # 26371, see page two for more information. One vial containing 1mg peptide lyophilized from 1ml sterile, distilled water.

Assay Dilution Buffer I (ADBI), Catalog # 20-108. Three vials, each containing 1.0ml of ADBI: 20mM MOPS, pH 7.2, 25mM β-glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

Magnesium/ATP Cocktail, Catalog # 20-113. One vial containing 1.0ml of Mg2+/ATP cocktail: 75mM magnesium chloride and 500µM ATP in ADBI.

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

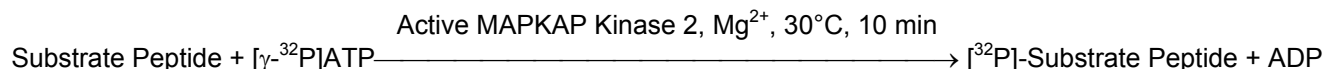
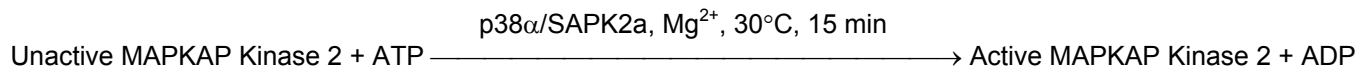
Kit Description

Quantity: 50 gel kinase assays per kit or 25 kinase assays using the substrate peptide. The kit contains sufficient positive control kinase p38α/SAPK2a MAPK for 100 assays.

Storage: Upon receipt, store individual components at recommended temperatures. Store component 14-210 at -70°C and component, 12-240 at 4°C. Store all other components at -20°C.

Stability: Components stable for 6 months from date of shipment if stored and handled correctly.

Use: The assay kit is designed to measure p38α/SAPK2a phosphotransferase activity in cell lysates, tissue homogenates or column fractions. After activation by p38α/SAPK2a, MAPKAP Kinase 2 can transfer the γ phosphate of [γ-32P]ATP to a specific peptide substrate. The phosphorylated substrate is then separated from the residual [γ-32P]ATP by differential binding to P81 phosphocellulose paper. After extensively washing the paper with phosphoric acid, the bound radioactivity is determined by liquid scintillation counting. The assay is linear for incubation times of up to 30 minutes and incorporation of up to 20% of total ATP. Longer incubation times or higher levels of incorporation may not be linear and therefore may not be a true indication of p38α/SAPK2a activity in the sample extract. This enzyme assay is rapid, convenient and specific for p38α/SAPK2a.



Technical Information for Kit Components

p38 α /SAPK2a, active (recombinant protein expressed in *E. coli*)

Product Description: Residues 4-361 of Xenopus p38 α /SAPK2a fused to the Mal-E fusion protein at the N-terminus linked by the sequence SerThr. The enzyme is purified by affinity chromatography on amylose beads followed by activation by partially purified rabbit MKK6.

Activity: 100 Units/ml by independent laboratory. 1 Unit of p38 α /SAPK2a = 1 Unit of MAPKAP Kinase 2 generated per minute.

Specificity: Essentially identical proteins in mammals (p38 MAP Kinases) and yeast (HOG-1). Inhibitors include protein phosphatase 2A, PTP-1B, CL-100 and pyridinyl imidazols.

Physical Form: Frozen solution.

MAPKAP Kinase 2, unactive (recombinant protein expressed in *E. coli*)

Product Description: Residues 46-400 of recombinant human MAPKAP Kinase 2 with an N-terminal GST tag and a C-terminal myc epitope. Partially purified to >60% by glutathione-agarose chromatography.

Specific Activity: As provided, this lot demonstrated <1% of maximum activity.

Physical Form: Frozen solution.

MAPKAP Kinase 2 Substrate Peptide

Background: This peptide is suitable as a substrate for MAPKAP Kinase 2 (Catalog #14-146) or MAPKAP Kinase 2, unactivated. The peptide can also be used as a substrate for p90 S6 kinase and CAM kinase II. Peptide sequence (KKLNRTLVA) is related to human glycogen synthase residues 1-9. MW=1129.

Description: Peptide (KKLNRTLVA) related to human glycogen synthase residues 1-9. MW=1129Da.

Purity: Greater than 95%.

References:

1. Zhong, Y., *et al.*, FEBS Lett. **275**: 20913-20923, 2001.
2. Song, S., *et al.*, Br. J. Haematol. **107**: 532-538, 1999.
3. Doza, Y.N., *et al.*, FEBS Lett. **364**: 223-228, 1995.
4. Ben-Levy, *et al.*, EMBO. J. **13**: 3302-3311, 1994.
5. Cuenda, A., *et al.*, FEBS Lett. **364**: 229-231, 1995.
6. Meier, R., *et al.*, Eur. J. Biochem. **236**: 796-805, 1996.
7. Stokoe, *et al.*, Biochem. **296**: 843, 1993.

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

- **stimulated cell or tissue extract containing active p38 α /SAPK2a**
- acetone
- vortex mixer
- plexiglass shielding
- 30°C shaking incubator
- timer
- variable volume (5-200 μ l) pipet + tips
- 0.75% phosphoric acid
- scintillation vials
- scintillation fluid
- scintillation counter
- [γ -³²P]ATP - ~3000 Ci/mmol, obtained from PerkinElmer, Cat. # BLU002A.

Safety Warnings and Precautions: The p38 α /SAPK2a kinase assay kit is designed for research use only and not recommended for internal use in humans or animals. Since the kit involves the use of radioactive [γ -³²P]ATP, follow your institutional rules for handling use, storage and disposal of radioactive materials. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.

p38 α /SAPK2a Kinase Cascade Assay Kit Procedures

The kit components should be thawed, mixed by vortexing and stored on ice before proceeding with the assay. In particular, Assay Dilution Buffer I (ADBI) and Magnesium/ATP cocktail must be rapidly thawed and mixed completely. Note: do not use extended thawing time. The assay components can be refrozen at -20°C for extended periods. Perform all pre-incubation steps at 1°C over an ice bath. The kinase assay may be performed at 30°C or room temperature but linear results are more easily achieved at 30°C. Activation of MAPKAP Kinase 2 is determined using the phosphorylation assay protocol described below. Activated MAPKAP Kinase 2 will phosphorylate the MAPKAP Kinase 2 substrate *in vitro*.

Kinase Assay Protocol

Stock Solutions:

1. Assay Dilution Buffer I (ADBI, Catalog # 20-108): 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2. Magnesium/ATP Cocktail (Catalog # 20-113): 500 μ M cold ATP and 75mM magnesium chloride in ADBI.
3. [γ -³²P]ATP: Stock 1mCi/100 μ l (3000Ci/mmol, obtained from PerkinElmer, Cat. # BLU002A). Make 10 μ l aliquots (100 μ Ci/vial). Before starting the assay, dilute an aliquot with 90 μ l of Magnesium/ATP cocktail
4. p38 α /SAPK2a, active (Catalog # 14-210): Dilute to 20ng/ μ l with ADBI containing 0.03% Brij-35 and 1mg/ml BSA. Use 3 μ l per assay point. .
5. MAPKAP Kinase 2 Substrate Peptide (Catalog # 12-240): Prepare a 0.86mM stock by rehydrating with 1028 μ l ADBI.
6. MAPKAP Kinase 2, unactive (Catalog # 14-216): Dilute to 40ng/ μ l with ADBI containing 0.03% Brij-35 and 1mg/ml BSA. .

Assay Procedure:

Stage One: Activation of MAPKAP Kinase 2

1. Add 5µl (200ng) of unactive MAPKAP Kinase 2.
2. Add 3µl (60ng) of either purified p38α/SAPK2a or a stimulated cell/tissue extract containing active p38α/SAPK2a.
3. Add 2µl Magnesium/ATP cocktail.
4. Incubate for 15 minutes at 30°C with agitation.

As an alternative to measuring incorporation of [³²P] into substrate peptide, the first stage phosphorylation reaction may be performed with the diluted [³²P]ATP. A sample of the radiolabeled reaction mixture may then be analyzed by a combination of SDS-PAGE and autoradiography to qualitatively determine p38α/SAPK2a activity by the visualization of phosphorylated MAPKAP Kinase 2.

Stage Two: Phosphorylation of MAPKAP Kinase 2 Substrate Peptide

1. Add 40µl of ice-cold ADBI to each tube prepared in stage one.
2. Add 10µl of diluted [³²P]ATP stock solution.
3. Add 10µl of 0.86mM stock MAPKAP Kinase 2 substrate peptide.
4. Incubate for 10 minutes at 30°C with agitation.
5. Transfer 40µl of this reaction on 2cm x 2cm P81 paper squares; see technical note below on using P81 paper.
6. Wash assay squares three times with 0.75% phosphoric acid for 5 minutes per wash.
7. Wash assay squares once with acetone for five minutes.
8. Transfer assay squares to a scintillation vial and add 5ml scintillation cocktail.
9. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).

Technical Note: Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.

p38α/SAPK2a Assay Kit Quality Control Data:

p38α/SAPK2a activity was measured using the kinase cascade assay described above. Assay results are shown to the right:

p38α/ SAPK2a	MAPKAP Kinase 2	Substrate Peptide	Mean CPM	Comments
60ng	none	123µM	9,714	Background
None	200ng	123µM	10,248	Background
60ng	200ng	123µM	320,501	p38α/SAPK2 a activity + background