Datasheet for ABIN625229

MAPK14 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MAPK14
Binding Specificity:	pThr180, pTyr182
Reactivity:	Human, Rat, Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human/Mouse Phospho-p38 alpha (T180/Y182) ELISA Kit. This assay semi-quantitatively measures phophorylated p38 alpha (Thr180/Tyr182) in lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human and mouse Phospho-P38 alpha (pThr180/pTyr182).
Characteristics:	• Rapidly measure phosphorylated protein in lysates • Screen numerous different cell lysates without performing a Western Blot analysis • Minimal hands-on time, convenient, and non-radioactive material
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Anti-Phospho Antibody

Product Details

- HRP-Conjugated Secondary Antibody
- Assay Diluent
- TMB One-Step Substrate
- Stop Solution
- Lysis Buffer
- Positive Control Sample

Material not included:

- Distilled or deionized water
- 100 mL and 1 liter graduated cylinders
- Tubes to prepare sample dilutions
- Protease and Phosphatase inhibitors
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- Benchtop rocker or shaker
- Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	MAPK14
Alternative Name:	p38 (MAPK14 Products)
Background:	P38-T180
Gene ID:	1432
UniProt:	Q16539
Pathways:	MAPK Signaling, Neurotrophin Signaling Pathway, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Regulation of Muscle Cell Differentiation, Regulation of Cell Size, Hepatitis C, Toll-Like Receptors Cascades, Autophagy, Thromboxane A2 Receptor Signaling, BCR Signaling, S100 Proteins

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents and samples as instructed in the manual. 2. Add 100 µL of sample or positive control to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared primary antibody to each well. 5. Incubate 1 h at RT.

6. Add 100 µL of prepared 1X HRP-Streptavidin to each well.
7. Incubate 1 h at RT.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use.

2. Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

3. Briefly spin the Positive Control vial of Item K. Add 400 µL 1x Assay Diluent (Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item K vial to prepare a Positive Control (P-1) Solution. Dissolve the powder thoroughly by a gentle mix (it can be removed by centrifuge if any precipitate in the solution is found. See i. Positive Control Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 6 of part IX.for a typical result in page 9). Pipette 240 µL 1x Assay Diluent into each tube. Use the Positive Control (P-1) to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background.

4. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

5. Briefly spin the detection antibody p38 alpha MAPK (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody should be diluted 55-fold with 1x Assay Diuent and used in step 4 of Part VII Assay Procedure.

6. Briefly spin the HRP-conjugated anti-rabbit IgG (Item D-1) before use. Pipette up and down to mix gently. HRP- conjugated anti-rabbit IgG concentrate should be diluted 1,000- P-1 P -2 P-3 P-4 0 120 µL Vial of Item K 400 µL 1x Assay Diluent 120µ l 120 µL Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 7 fold with 1x Assay Diuent. For example: Briefly spin the vial (ItemD) and pipette up and down to mix gently. Add 10 µL of HRP-conjugated anti- rabbit IgG concentrate into a tube with 10 mL 1x Assay Diluent to prepare a 1,000-fold diluted HRP-conjugated anti-rabbit IgG solution.

7. Cell Lysate Buffer should be diluted 2-folds with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). VII.

Sample Preparation:

Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4 x 107 cells/mL in 1x Cell Lysate Buffer (we recommend adding protease and phosphatase inhibitors to Cell Lysate Buffer prior to sample preparation). Pipette up and down to resuspend and incubate the lysates with shaking at 2 - 8° C for 30

	minutes. Microcentrifuge at 13,000 rpm for 10 minutes at 2 - 8° C, and transfer the supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at -70 °C. Avoid repeated Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 5 freeze-thaw cycles. Thawed lysates should be kept on ice prior to use. For the initial experiment, we recommend to do a serial dilution testing such as 5-fold and 100-fold dilution for your cell lysates with 1x Assay Diluent (Item E) before use. Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empirically. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors).
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate. 2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 8 4. Add 100 µL of prepared 1X anti-p38 alpha MAPK antibody (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. 5. Discard the solution. Repeat the wash as in step3. 6. Add 100 µL of prepared 1X HRP-conjugated anti-rabbit IgG solution (see Reagent Preparation step 6) to each well. Incubate for 2 hour at room temperature with shaking. 7. Discard the solution. Repeat the wash as in step3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	ELISA data analysis: Average the duplicate readings for each sample or positive control. i. Positive Control Hela cells were treated with Anisomycin at 37 °C for 10 min. Solubilize cells at 4 x 107 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI Reagent Preparation for detail. Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 10 Assay Diluent Positive control dilution series O D = 4 5 0 n m 0.01 0.1 1 10 P-1 P-2 P-3 P-4

Application Details

	ii. Anisomycin Stimulation of Hela Cell Lines Hela cells were treated or untreated with Anisomycin for 10 min at 37 °C. Cell lysates were analyzed using this phosphoELISA and Western Blot. Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 11 A). ELISA p38 alpha MAPK (Thr180/Tyr182) pan p38 alpha MAPK OD =4 50 n m 0.0 0.5 1.0 1.5 Untreated Hela Anisomycin treated Hela B). Western-Blot Analysis Anisomycine 0 10 0 10 (Min) Anti-phospho-p38 alpha MAPK Anti-pan p38 MAPK (Thr180/Tyr182) Phospho-p38 alpha MAPK (Thr180/Tyr182) ELISA Kit Protocol 12 X
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze- thaw cycles.
Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months

Publications

Product cited in:	Meireles, Marques, Norberto, Fernandes, Mateus, Rendeiro, Spencer, Faria, Calhau: "The impact of chronic blackberry intake on the neuroinflammatory status of rats fed a standard or high-fat diet." in: **The Journal of nutritional biochemistry**, Vol. 26, Issue 11, pp. 1166-73, (2015) (PubMed). Hanaoka, Nicolls, Fontenot, Kraskauskas, Mack, Kratzer, Salys, Kraskauskiene, Burns, Voelkel, Taraseviciene-Stewart: "Immunomodulatory strategies prevent the development of autoimmune emphysema." in: **Respiratory research**, Vol. 11, pp. 179, (2010) (PubMed).

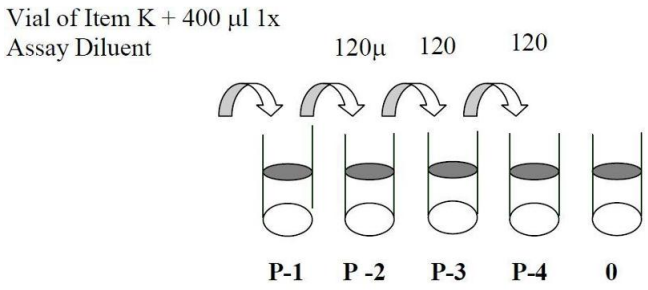

Image 1. This picture shows the preparation of the positive control.

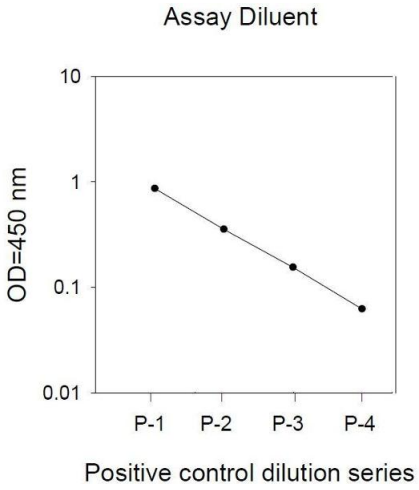

Image 2.

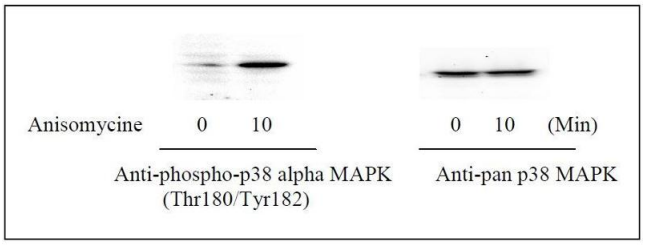

Image 3.

Please check the product details page for more images. Overall 4 images are available for ABIN625229.