

## Product Information

**Cell-Based ELISA Sampler Kit for detecting phospho-ERK1/2 (pThr<sup>202</sup>/pTyr<sup>204</sup>), phospho-JNK (pThr<sup>183</sup>/pTyr<sup>185</sup>), and phospho-p38 MAPK (pThr<sup>180</sup>/pTyr<sup>182</sup>) in cultured cell lines**  
adequate for 192 assays (2 × 96 well plate)

Catalog Number **RAB0352**  
Storage Temperature -20 °C

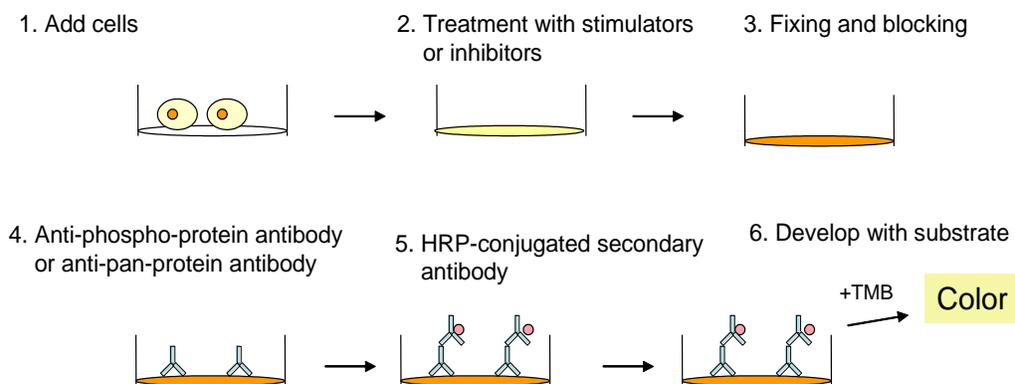
## TECHNICAL BULLETIN

### Product Description

Protein phosphorylation is instrumental in the regulation of protein activity within a cell. It plays important roles in the living cells including proliferation, differentiation, and metabolism. A large number of protein kinases and phosphatases have been extensively investigated, and have been shown to be involved in signal transduction pathways.

The Cell-Based ELISA Sampler Kit is a very rapid, convenient, and sensitive assay kit that can monitor the activation or function of important biological pathways in cells (see Figure 1). It can be used for measuring the relative amount of ERK1/2 (pThr<sup>202</sup>/pTyr<sup>204</sup>), JNK (pThr<sup>183</sup>/pTyr<sup>185</sup>), and p38 MAPK (pThr<sup>180</sup>/pTyr<sup>182</sup>) phosphorylation, and screening the effects of various inhibitors (such as siRNA or chemicals) or activators in cultured human, mouse, and rat cell lines.

By determining specific protein phosphorylation in the experimental model system, pathway activation can be verified in the cell lines without spending time and effort in preparing a cell lysate and performing Western blot analysis. In the Cell-Based ELISA Sampler Kit, cells are seeded into a 96-well tissue culture plate. The cells are fixed after various treatments, such as inhibitors or activators. After blocking, anti-phosphoprotein specific antibody or anti-pan-protein specific antibody (primary antibody) is pipetted into the wells and incubated. The wells are washed, and HRP-conjugated anti-mouse IgG (secondary antibody) is added to the wells. The wells are washed again, a TMB substrate solution is added to the wells and color develops in proportion to the amount of protein. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.



**Fig.1. Cell-Based protein phosphorylation procedure**

## Components

1. 2 Uncoated Microplates (Item A) - RABPLATE1: two 96 well tissue culture plates (12 × 8 wells) for cell culture.
2. 20x Wash Buffer Concentrate A (Item B): - RABWASH1 30 mL of 20x concentrated buffer.
3. 20x Wash Buffer Concentrate B (Item C) - RABWASH2: 30 mL of 20x concentrated buffer.
4. Fixing Solution (Item D) - RABFIX1: 30 mL of fixing solution.
5. Quenching Solution for Cell-based ELISA Assay (Item E) - RABQUENCH: 2 mL of 30x concentrated solution.
6. 5x Blocking Solution (Item F) - RABBLOCK: 20 mL of 5x concentrated solution.
7. Phospho-specific Antibody Concentrate (Item G1) - RABE204G: one tube (30 assays) of anti-phospho-ERK1/2 (pThr<sup>202</sup>/pTyr<sup>204</sup>).
8. Pan ERK Antibody Concentrate (Item H1) - RABERKH: one tube (30 assays) of anti-ERK1/2.
9. Phospho-specific Antibody Concentrate (Item G2): one tube (30 assays) of anti-phospho-p38 MAPK (pThr<sup>180</sup>/pTyr<sup>182</sup>).
10. Pan p38 Antibody Concentrate (Item H2): one tube (30 assays) of anti-p38 MAPK.
11. Phospho-specific Antibody Concentrate (Item G3): one tube (30 assays) of anti-phospho-JNK (pThr<sup>183</sup>/pTyr<sup>185</sup>).
12. Pan JNK Antibody Concentrate (Item H3): one tube (30 assays) of anti-JNK.
13. HRP-conjugated Anti-Mouse IgG Concentrate (Item I) - RABHRP1: two tubes (192 assays) 10 μL of 1,000x concentrated HRP-conjugated anti-mouse IgG.
14. TMB Substrate Reagent (Item J) - RABTMB1: two bottles (192 assays) 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
15. Stop Solution (Item K) - RABSTOP1: 14 mL of sulfuric acid.

## Reagents and Equipment Required but Not Provided.

1. A model cell line, Protein tyrosine kinase inhibitors, growth factor or cytokine.
2. Microplate reader capable of measuring absorbance at 450 nm.
3. 37 °C incubator.
4. Precision pipettes to deliver 2 μL to 1 mL volumes.
5. Adjustable 1-25 mL pipettes for reagent preparation.
6. 100 mL and 1 liter graduated cylinders.
7. Absorbent paper.
8. Distilled or deionized water.
9. Poly-L-lysine solution, Catalog Number P4832.

## Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

1. Wash Buffer A (20x) (Item B) or B (20x) (Item C) should be diluted 20-fold with deionized or distilled water. If the Wash Buffer A (20x) or B (20x) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 25 mL of Wash Buffer Concentrate into deionized or distilled water to yield 500 mL of 1x Wash Buffer.
2. Quenching Buffer Concentrate (Item E): should be diluted 30-fold with 1x Wash Buffer A before use.
3. Blocking Solution (5x) (Item F): should be diluted 5-fold with deionized or distilled water.
4. Preparation of antibody: Briefly spin the antibody tubes (Items G1, G2, G3, H1, H2, and H3) before use. Add 100 mL of 1x Blocking Solution into each tube to prepare each primary antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at –20 °C for 1 month, avoid repeated freeze-thaw cycles). Each antibody concentrate should be diluted 15-fold with 1x Blocking Solution before use.
5. HRP-conjugated Anti-Mouse IgG Concentrate (Item I): should be diluted 1,000-fold with 1x Blocking Solution before use (Briefly spin the tube, Item I before use).

## Storage/Stability

Upon receipt, the kit should be stored at –20 °C. Please use within 6 months from the date of shipment.

Items B, D, E, F, J, and I should be stored at 2–8 °C to avoid repeated freeze-thaw cycles after initial use.

Item I store at 2–8 °C for up to one month (store at –20 °C for up to 6 months, avoid repeated freeze-thaw cycles).

Items G1, G2, G3, H1, H2, and H3 should be stored at –20 °C after use.



4. Add 100  $\mu\text{L}$  of Fixing Solution (Item D) into each well and incubate for 20 minutes at room temperature with shaking.
5. Wash the plate 3 times with 1x Wash Buffer A, then tap the plate upside down to remove all of wash buffer.
6. Add 200  $\mu\text{L}$  of prepared 1x Quenching Buffer (Item E) and incubate 20 minutes at room temperature.
7. Wash the plate 4 times with 1x Wash Buffer A, then tap the plate upside down to remove all of wash buffer.
8. Add 200  $\mu\text{L}$  of prepared 1x Blocking Solution (Item F) and incubate for 1 hour at 37 °C.
9. Wash 3 times with 1x Wash Buffer B (200  $\mu\text{L}$  each), then tap the plate upside down to remove all of excess wash buffer.

**Note:** The plate may be stored at  $-70\text{ }^{\circ}\text{C}$  for several days.

10. Add 50  $\mu\text{L}$  of 1x anti-phosphoprotein specific antibody (Item G1, G2, or G3) or anti-pan-protein specific antibody (Item H1, H2, or H3) to the corresponding well and incubate for 2 hours at room temperature with shaking.
11. Wash 4 times with 1x Wash Buffer B (200  $\mu\text{L}$  each), then tap the plate upside down to remove all of excess wash buffer.
12. Add 50  $\mu\text{L}$  of 1x HRP-conjugated Anti-Mouse IgG (Item I) and incubate for 1 hour at room temperature.
13. Wash 4 times with 1x Wash Buffer B (200  $\mu\text{L}$  each), then tap the plate upside down to remove all of excess wash buffer.
14. Add 100  $\mu\text{L}$  of TMB Substrate Reagent to each well and incubate for 30 minutes with shaking at room temperature in the dark.
15. Add 50  $\mu\text{L}$  of stop solution to each well and read at 450 nm, measure OD immediately.

## Results

Representative results are shown:

### Note:

1. In Procedure, step 2, A431 cells were seeded for measuring ERK1/2 and Hela cells were seeded for measuring JNK and p38 MAPK.

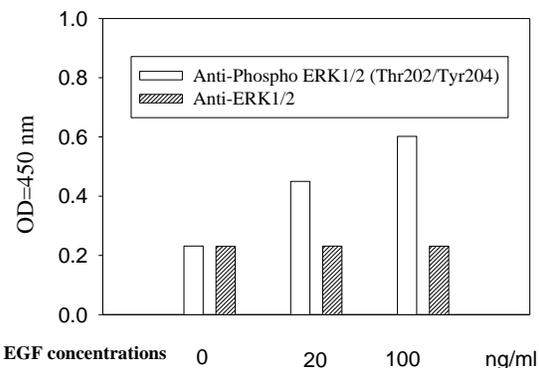
2. In Procedure, step 3, added 50  $\mu\text{L}$  of stimulator solutions with different concentrations:

A431 cells: 0, 20, or 100 ng/mL of rhEGF in serum free DMEM

Hela cells: 0, 0.2, or 1  $\mu\text{g}/\text{mL}$  of anisomycin in serum free DMEM)

Then incubate cells for 10–60 minutes at 37 °C.

3. Discarded the solution and washed 3 times with 1x Wash Buffer A (200  $\mu\text{L}$  each) immediately. Then tapped the plate upside down to remove all of excess wash buffer and followed with Procedure, steps 4–15.



**Fig. 3.1** A431 cells were stimulated by different concentrations of EGF for 10 min at 37°C.

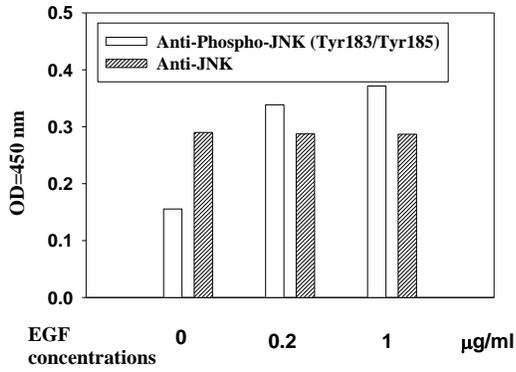


Fig. 3.2 HeLa cells were stimulated by different concentrations of anisomycin for 1 hour at 37°C

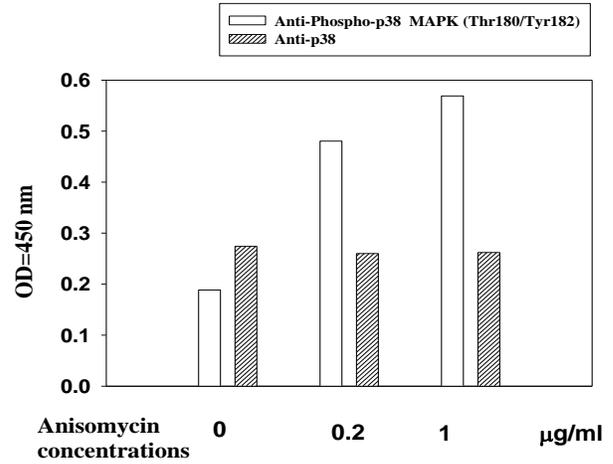


Fig. 3.3 HeLa cells were stimulated by different concentrations of anisomycin for 1 hour at 37°C

## Western blots

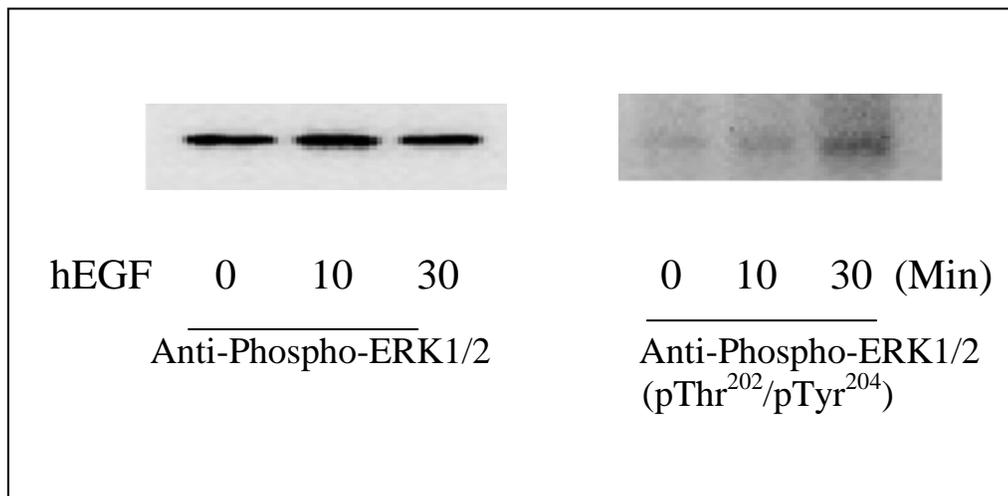
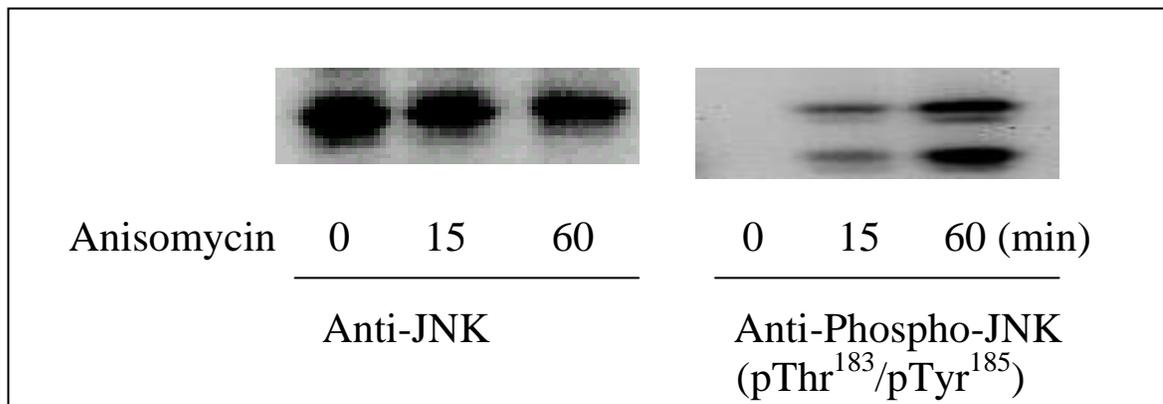
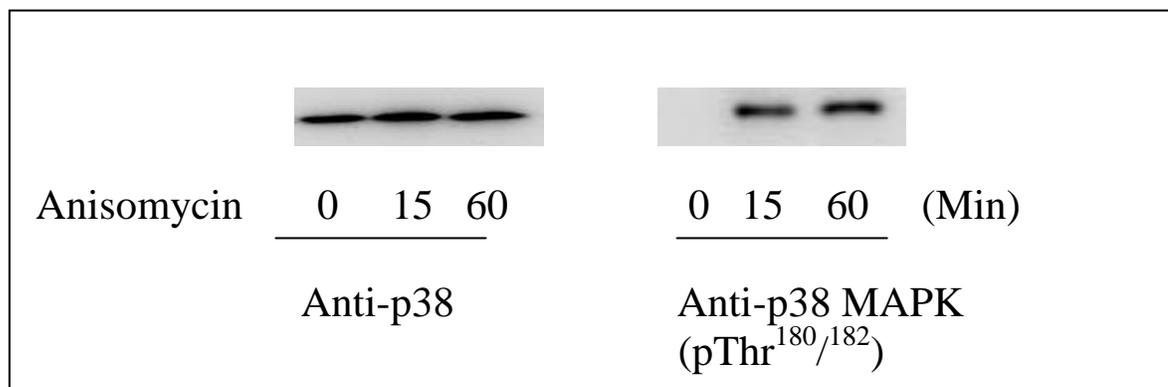


Figure 4.1. Western blot analysis of extracts from 100 ng/mL hEGF treated A431 cells. Phospho-ERK1/2 (pThr<sup>202</sup>/pTyr<sup>204</sup>) and ERK1/2 antibodies were used in both detection assays.



**Figure 4.2.**

Western blot analysis of extracts from 1  $\mu\text{g/mL}$  Anisomycin treated HeLa cells. Anti-Phospho-JNK (pThr<sup>183</sup>/pTyr<sup>185</sup>) and Anti-JNK antibodies were used in both detection assays.



**Figure 4.3.**

Western blot analysis of extracts from 1  $\mu\text{g/mL}$  Anisomycin treated HeLa cells. Phospho-p38 MAPK (Thr<sup>180</sup>/Tyr<sup>182</sup>) and Anti-p38 MAPK antibodies were used in both detection assays.

#### References

1. Winston, B.W. et al., J. Immunol., **159**, 4491-4497 (1997).
2. Michael, J. et al., Protein Phosphorylation in Cell Growth Regulation. 1st Edition (1997).
3. Clark, E.A., and Hynes, R.O., J. Biol. Chem., **271**, 14814 (1996).

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**Appendix**  
Troubleshooting Guide

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Low signal	Improper storage of the ELISA kit	Store all of components according to manual instructions. Keep TMB substrate solution in dark
	Improper dilution	Ensure correct preparation of antibody and reagents
	Cells drop off from the wells	Some of treatments may make cells drop off from the wells. Reduce inhibitor or activator concentration.
High background	Inadequate washing	Be sure to remove all of washing solution and follow the recommendation for washing
	Too many cells	Reduce the cell number
Large CV	Inaccurate pipetting	Check pipette
	Remaining wash buffer in the well	Remove all of wash buffer
	Cells drop off from the wells	Please don't directly contact the cells with tips when adding reagents or wash buffer.

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