Revision 1

#11975 Store at +4C

PathScan® Phospho-FRA1 (Ser265) Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity: H

UniProt ID: #P15407

Entrez-Gene Id: #8061

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
TMB Substrate	7004	11 ml		+4C
STOP Solution	7002	11 ml		+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellow	-20C

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The PathScan® Phospho-FRA1 (Ser265) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of FRA1 when phosphorylated at Ser265. A Phospho-FRA1 (Ser265) Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, phosphorylated FRA1 protein is captured by the coated antibody. Following extensive washing, a biotinylated FRA1 Rabbit Detection mAb is added to detect the captured phospho-FRA1 (Ser265) protein. HRP-linked streptavidin is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for the developed color is proportional to the quantity of FRA1 phosphorylated at Ser265.
Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

PathScan® Phospho-FRA1 (Ser265) Sandwich ELISA Kit recognizes endogenous levels of FRA1 protein when phosphorylated at Ser265, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

The Fos family of nuclear oncogenes includes c-Fos, FosB, Fos-related antigen 1 (FRA1), and Fos-related antigen 2 (FRA2) (1). While most Fos proteins exist as a single isoform, the FosB protein exists as two isoforms: full-length FosB and a shorter form, FosB2 (Delta FosB), which lacks the carboxy-terminal 101 amino acids (1-3). The expression of Fos proteins is rapidly and transiently induced by a variety of extracellular stimuli, including growth factors, cytokines, neurotransmitters, polypeptide hormones, and stress. Fos proteins dimerize with Jun proteins (c-Jun, JunB, and JunD) to form Activator Protein-1 (AP-1), a transcription factor that binds to TRE/AP-1 elements and activates transcription. Fos and Jun proteins contain the leucine-zipper motif that mediates dimerization and an adjacent basic domain that binds to DNA. The various Fos/Jun heterodimers differ in their ability to transactivate AP-1 dependent genes. In addition to increased expression, phosphorylation of Fos proteins by Erk kinases in response to extracellular stimuli may further increase transcriptional activity (4-6). Phosphorylation of c-Fos at Ser32 and Thr232 by Erk5 increases protein stability and nuclear localization (5). Phosphorylation of FRA1 at Ser252 and Ser265 by Erk1/2 increases protein stability and leads to overexpression of FRA1 in cancer cells (6). Following growth factor stimulation, expression of FosB and c-Fos in quiescent fibroblasts is immediate, but very short-lived, with protein levels dissipating after several hours (7). FRA1 and FRA2 expression persists longer, and appreciable levels can be detected in asynchronously growing cells (8). Deregulated expression of c-Fos, FosB, or FRA2 can result in neoplastic cellular transformation; however, Delta FosB lacks the ability to transform cells (2,3).

Background References

1. Tulchinsky, E. (2000) *Histol Histopathol* 15, 921-8.
2. Dobrazanski, P. et al. (1991) *Mol Cell Biol* 11, 5470-8.
3. Nakabeppu, Y. and Nathans, D. (1991) *Cell* 64, 751-9.
4. Rosenberger, S.F. et al. (1999) *J Biol Chem* 274, 1124-30.
5. Sasaki, T. et al. (2006) *Mol Cell* 24, 63-75.
6. Basbous, J. et al. (2007) *Mol Cell Biol* 27, 3936-50.

7. Kovary, K. and Bravo, R. (1991) *Mol Cell Biol* 11, 2451-9.
8. Kovary, K. and Bravo, R. (1992) *Mol Cell Biol* 12, 5015-23.

Trademarks and Patents

Limited Uses

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ELISA Colorimetric

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808) To prepare 1 L PBS: add 50 ml 10X PBS to 950 ml dH_2O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH_2O.
4. **1X Cell Lysis Buffer**: 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH_2O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

   Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

   NOTE: Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate**: (#7004).
6. **STOP Solution**: (#7002).

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

C. Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed in the storage bag and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. **Gently remove the tape and wash wells**:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X wash buffer, 200 µl each time per well.
    3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
    4. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of detection antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.

6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 µl of STOP solution to each well. Shake gently for a few seconds.

    NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP solution.

11. **Read results**
    1. **Visual Determination**: Read within 30 min after adding STOP solution.
    2. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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