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Product Information

Phosphotyrosine FER/Tyrosine-protein Kinase Fer ELISA Kit
for Measuring Phosphorylated FER (phosphotyrosine protein) in human cell lysates

Catalog Number **RAB0960**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Phosphotyrosine FER ELISA Kit is a very rapid, convenient, and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated FER in the experimental model system, pathway activation can be verified in the cell lysates. One can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western blot analysis.

This sandwich-based ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the measurement of human phospho-FER. An anti-FER antibody has been coated onto a 96-well plate. Samples are pipetted into the wells, and phosphorylated and non-phosphorylated FER present in a sample are bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-phosphotyrosine antibody is used to detect only tyrosine-phosphorylated protein. After washing away unbound antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of phospho-FER bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Human Phosphotyrosine FER Antibody-coated ELISA Plate (Item A): 96 wells (12 strips × 8 wells) coated with anti-pan-FER.
2. Phosphotyrosine FER Positive Control, Lyophilized (Item K): 1 vial of lyophilized powder from Jurkat cell lysate.
3. Biotinylated Anti-Human Phosphotyrosine Antibody (Item C): 2 vials of biotinylated anti-phospho-tyrosine (each vial is enough to assay half microplate).
4. 20x Wash Buffer Concentrate (Item B): 25 mL of 20x concentrated solution.
5. Phosphotyrosine ELISA HRP-Streptavidin (Item G): 1 vial (200 µL) of 600x concentrated HRPconjugated streptavidin.
6. TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffer solution.
7. Phosphorylation ELISA Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.
8. 5x Assay Diluent (Item E2): 15 mL of 5x concentrated buffer. For diluting cell lysate samples, detection antibody (Item C), and HRP-Streptavidin concentrate.
9. 2x Cell Lysate Buffer (Item J): 10 mL of 2x cell lysis buffer (does not include protease and phosphatase inhibitors).

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Protease and Phosphatase inhibitors.
3. Shaker.
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. Log-log graph paper or computer and software for ELISA data analysis.
10. Tubes to prepare the positive control or sample dilutions.

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

Sample Preparation

2x Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water to yield 1x Cell Lysate Buffer (addition of protease and phosphatase inhibitors to 1x Cell Lysate Buffer is recommended prior to sample preparation).

Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4×10^7 cells/mL in 1x Cell Lysate Buffer. 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 supernatants into a clean test tube. Lysates should be used immediately, or aliquoted and stored at –70 °C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

For the initial experiment, it is recommend to do serial dilution testing such as 5-fold and 100-fold dilution for the cell lysates with Assay Diluent (Item E2) 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.

Reagent Preparation

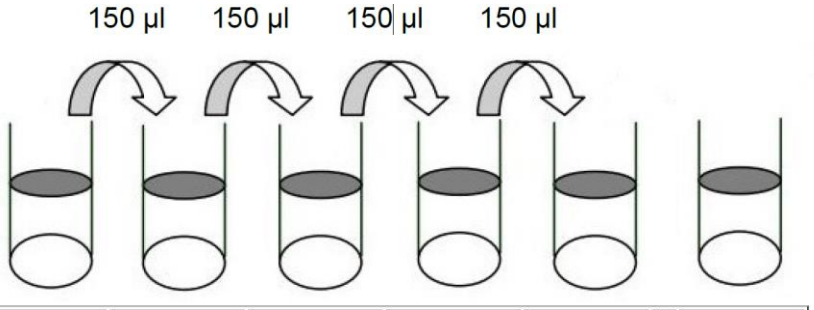
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. 5x Assay Diluent (Item E2), should be diluted 5-fold with deionized or distilled water before use.

3. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate). The addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use is recommended.

4. Preparation of Positive Control: Briefly spin the Positive Control Vial (Item K). Add 400 µL of prepared 1x Assay Diluent (Item E2) into Item K to prepare a Positive Control (P-1) solution. Gently mix the powder to allow it to dissolve thoroughly. If a precipitate is seen in the solution after mixing, this can be removed by a quick centrifugation of the positive control vial, and then pipetting the supernatant only for the assay. Pipette 300 µL of 1x Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the blank (P-0).

Figure 1.
Dilution Series for Positive Control



	P-1	P-2	P-3	P-4	P-5		Blank (P-0)
Diluent volume	Item K + 400 µl	300 µl	300 µl	300 µl	300 µl		300 µl

5. 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.

6. Preparation of Biotinylated anti-phosphotyrosine: Briefly spin the vial of Biotinylated anti-phospho-tyrosine (Item C). Add 100 µL of 1x Assay Diluent into the vial to prepare a phosphotyrosine detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days or at –70 °C for one month). The

concentrate should then be diluted 80-fold with 1x Assay Diluent and used in Procedure, step 4.

7. Preparation of HRP-Streptavidin: Briefly spin the vial of HRP-Streptavidin concentrate (Item G) before use. HRP-Streptavidin should be diluted 600-fold with 1x Assay Diluent and used in Procedure, step 7.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 12 mL of 1x Assay Diluent to prepare a 600-fold diluted HRP Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability

Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is strongly recommended to run all positive controls and samples in at least duplicate.

2. Label removable 8-well strips as appropriate for the experiment.

3. Add 100 µL of positive control (see Reagent Preparation step 4) or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel pipette or autowasher. Complete removal of liquid at each step is essential for 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.

5. Add 100 µL of prepared 1x biotinylated anti-phosphotyrosine (see Reagent Preparation, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 4.

6. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 4.

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 gentle shaking.

9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

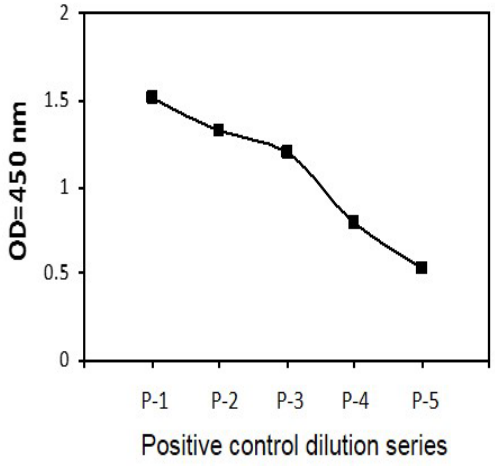
Results

Typical Data

Calculate the mean absorbance for each set of duplicate positive controls and samples, and then subtract the average zero (blank) optical density.

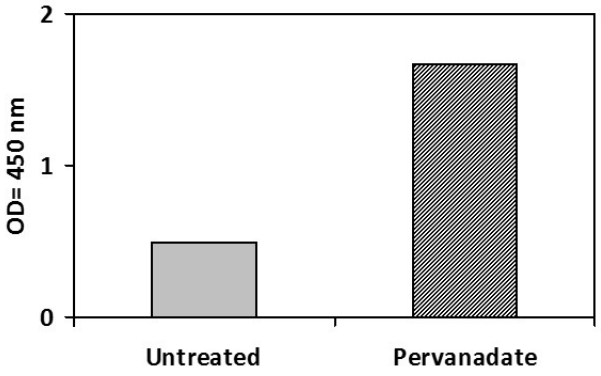
Positive Control

Jurkat cells were treated with pervanadate. Solubilize cells at 4×10^7 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed with this ELISA. See Reagent Preparation, step 3 for detail.



Pervanadate (PV) Stimulation of Jurkat Cell Line

Jurkat cells were treated with pervanadate. Cell lysates were analyzed using this phosphoELISA.


2
1
0
OD= 450 nm
Untreated
Pervanadate

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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