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Product Information

Phosphotyrosine TEC/Tyrosine-protein Kinase Tec ELISA Kit

Catalog Number **RAB1000**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

This sandwich-based ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the measurement of Human phospho-TEC. An anti-TEC antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and phosphorylated and unphosphorylated TEC present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated antiphosphotyrosine 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-TEC 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 TEC Antibody-coated ELISA Plate (Item A): 96 wells (12 strips × 8 wells) coated with anti-panTEC.
2. Phosphotyrosine TEC Positive Control, Lyophilized (Item K): 1 vial of lyophilized powder from K562 cell lysates.
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: 1 vial (200 µL) of 300x concentrated HRP-conjugated 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 E): 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. Distilled or deionized water.
8. Tubes to prepare 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 1x 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.

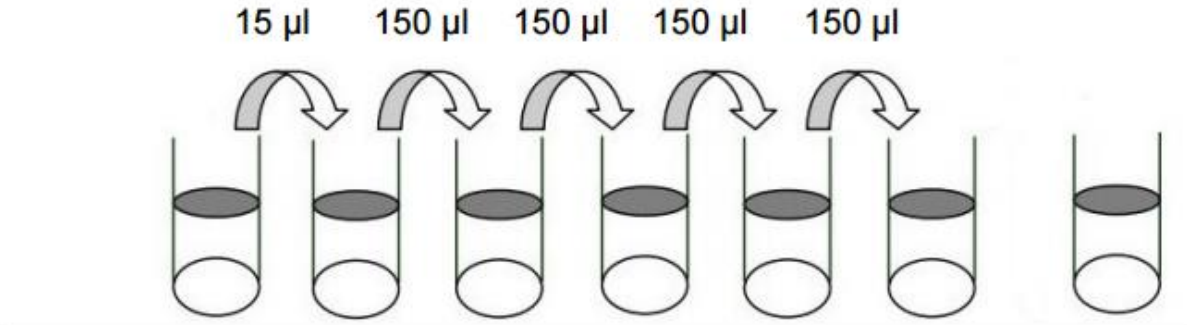
For the initial experiment, it is recommend to do serial dilution testing such as 5-fold and 50-fold dilution for the cell lysates with Assay Diluent (Item E) before use.

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.

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. 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 1,000 µL of prepared 1x Assay Diluent (Item E2) into Item K to prepare a Positive Control Stock 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 centrifuge of the positive control vial, and then pipetting the supernatant only for the assay. Add 15 µL of the prepared Positive Control Stock Solution from the vial of Item K into a tube with 435 µL of 1x Assay Diluent to prepare Positive Control (P-1). Pipette 300 µL of 1x Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the blank (P-0)

Figure 1.
Dilution Series for Positive Control



	Stock	P-1	P-2	P-3	P-4	P-5	Blank (P-0)
Diluent volume	Item K + 1000 µl	435 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 –80 °C for one month). The concentrate should then be diluted 80-fold with 1x Assay Diluent and used in step 4 of the Assay Procedure.

7. Preparation of HRP-Streptavidin: Briefly spin the vial of HRP-Streptavidin concentrate (Item G) before use. HRP-Streptavidin should be diluted 300-fold with 1x Assay Diluent and used in Procedure, step 6.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 mL of 1x Assay Diluent to prepare a 300-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 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 overnight 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 multichannel 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.

4. 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 3.

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 3.

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.

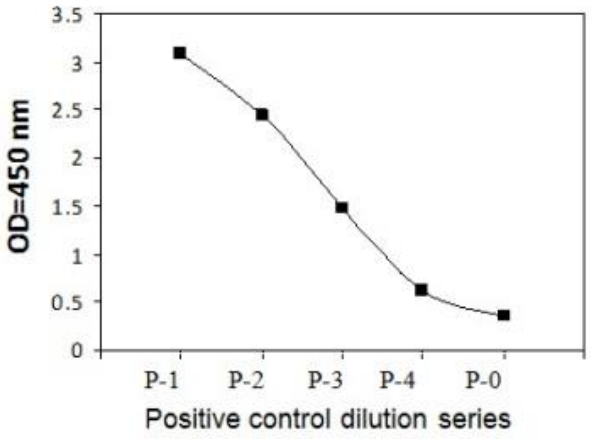
Results

Typical Data

ELISA data analysis: Average the duplicate readings for each sample or positive.

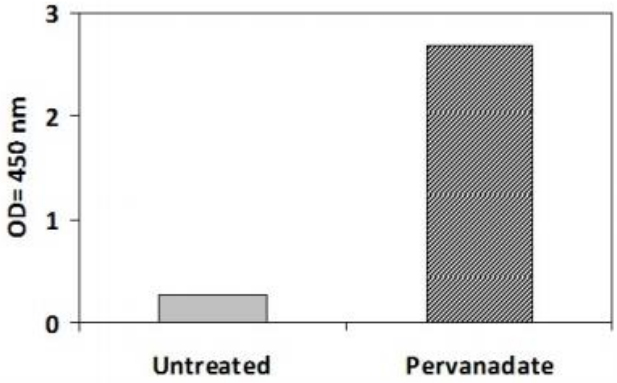
1. Positive Control

   K562 cells were treated with pervanadate at 37 °C for 10 min. Cells were solubilzed at 4 x 10^7 cells/mL in lysis buffer. Serial dilutions of lysates were analyzed with this ELISA kit (see Reagent Preparation, step 4).



2. Pervanadate Stimulation of K562 Cell Line

   K562 cells were untreated or treated with pervanadate for 10 min at 37 °C. Cell lysates were analyzed using this phospho-ELISA kit:



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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