3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Phosphotyrosine TYRO3/Tyrosine-protein Kinase Receptor TYRO3 ELISA Kit

Catalog Number **RAB0940**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

This Phosphotyrosine TYRO3 (DTK)/Tyrosine-protein Kinase Receptor TYRO3 ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the measurement of human phospho-DTK. An anti-DTK antibody has been coated onto a 96 well plate. Samples are pipetted into the wells, and phosphorylated and unphosphorylated DTK present in a sample is 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, HRPconjugated 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-DTK bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. DTK Microplate (Item A): 96 wells (12 strips × 8 wells) coated with anti-DTK.
2. Wash Buffer Concentrate (20x) (Item B): 25 mL of 20x concentrated solution.
3. Assay Diluent (Item E2): 15 mL of 5x concentrated buffer. For diluting cell lysate sample, detection antibody (Item C), and HRPStreptavidin Concentrate (Item G).
4. Biotinylated anti-phosphotyrosine (Item C): 2 vials of biotinylated anti-phosphotyrosine (each vial is enough to assay half microplate).
5. HRP-Streptavidin Concentrate (Item G): 1 vial, 200 µL/vial, 300x concentrated HRP-conjugated streptavidin.
6. TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3′,5,5′- tetramethylbenzidine (TMB) in buffered solution.
7. Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.
8. Cell Lysate Buffer (Item J): 5 mL of 2x cell lysis buffer (not including protease and phosphatase inhibitors).
9. Positive Control (Item K): 1 vial of lyophilized powder from cell lysates.

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 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 recommended to perform serial dilution testing such as 5-fold and 50-fold dilution for the cell lysates with 1x Assay Diluent (Item E2, diluted 5-fold with deionized or distilled water before use) 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.

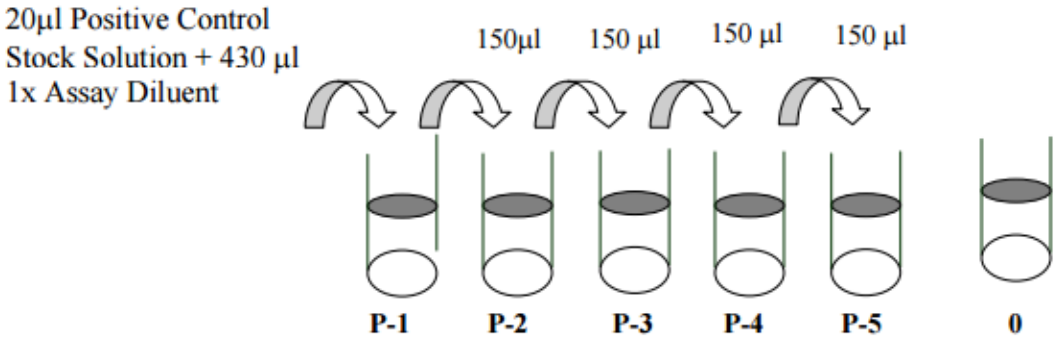
Reagent Preparation

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

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

3. Preparation of Positive Control: Briefly spin the Positive Control vial (Item K). Add 400 µL of 1x Assay Diluent (Item E2, 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. Pipette 300 µL of 1x Assay Diluent into each tube. Add 150 µL of prepared Positive Control P-1 into a tube with 300 µL of 1x Asaay Diluent to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background.

Figure 1.
Dilution Series for Positive Control



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 biotinylated antibody (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a biotinylated anti-phosphotyrosine 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 biotinylated phosphotyrosine antibody should be diluted 80-fold with 1x Assay Diluent and used in Procedure, step 4.

6. Briefly spin the HRP-Streptavidin concentrate vial (Item G), and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent. 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 1x Assay Diluent to prepare a 300-fold diluted HRP Streptavidin solution (don't store the diluted solution for next day use). Mix well.

7. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use. Addition of protease and phosphatase inhibitors is recommended.

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 Buffer. Wash by filling each well with 1x 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 antibody (Preparation, step 5) to each well. Incubate for 1 hour at room temperature with shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µL of prepared 1x HRP-Streptavidin solution (Preparation, step 6) to each well. Incubate for 45 minutes at room temperature with 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.

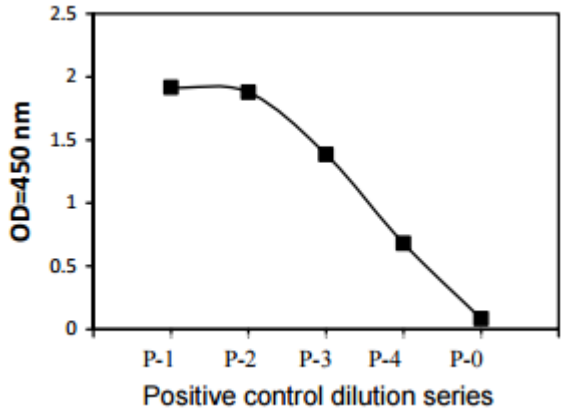
Results

Typical Data

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

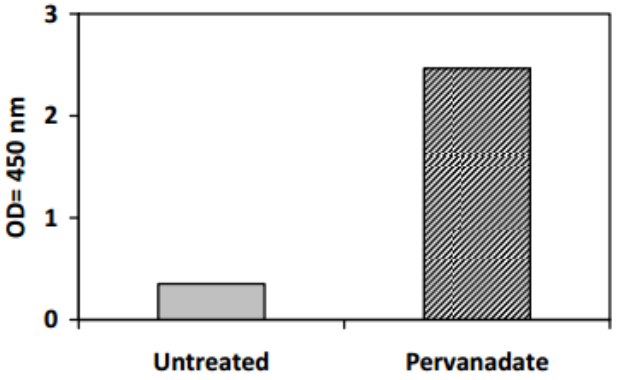
Positive Control:

U937 cells were treated with pervanadate at 37 °C for 10 minutes. Solubilize cells at 4×10^7 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see Preparation, step 3 for detail.



Pervanadate Stimulation of U937 Cell Line:

U937 cells were treated or untreated with pervanadate for 10 minutes at 37 °C. Cell lysates were analyzed using this phosphoELISA:



Appendix

Troubleshooting Guide

Problem	Cause	Solution
Sample signals are too low	Sample concentration is too low	Increasing sample concentration
Sample signals are too high	Sample concentration is too high	Reducing sample concentration
High background	Plate is insufficiently washed	Review the manual for proper washing. If using an automated plate washer, check that all ports are unobstructed.
	Contaminated Wash Buffer	Make fresh Wash Buffer
Large CV	Inaccurate pipetting	Check pipettes
Low positive control signal	Improper storage of the ELISA kit	Upon receipt, the kit should be stored at –20 °C. Store the positive control at –70 °C after reconstitution.
	Stop solution	Stop solution should be added to each well before measurement and read OD immediately.
	Improper primary or secondary antibody dilution	Ensure correct dilution

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