RayBio® Human, Mouse and Rat Phospho-P53 (Ser15) and Total P53 ELISA Kit

Catalog #: PEL-P53-S15-T

User Manual
Last revised March 23, 2021

Caution:
Extraordinarily useful information enclosed

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RayBiotech, Inc.

RayBio® Human, Mouse and Rat Phospho-P53 (Ser15) and Total P53 ELISA Kit

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Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

RayBio® Phospho-P53 (Ser15) and Pan P53 ELISA (Enzyme-Linked Immunosorbent Assay) 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 P53 protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blot analysis.

This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of Human, Mouse and Rat P53 (Ser15) and pan P53. The left side of a 96-well plate is coated with anti-P53 (Ser15) (distinguished by red marker) and the right side of the plate is coated with anti-pan P53 antibody (distinguished by black marker). Samples are pipetted into the wells and phosphorylated P53 (left side) and pan P53 (right side) present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-pan-P53 is used to detect phosphorylated P53 (Ser15) or pan P53. After washing away unbound antibody, HRP-Streptavidin is pipetted into 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 P53 (Ser15) or pan P53 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. STORAGE

The entire kit may be stored at -20°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

III. REAGENTS

Component	Size / Description	Storage / Stability After Preparation
P53 Microplate (Item A)	96 wells (12 strips x 8 wells). Six strips, marked with red marker (provided on the left), are coated with anti-phospho-P53 (Ser15). The remaining 6 strips, marked with black marker (provided on the right), are coated with anti-pan-P53.	1 month at -20°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Positive Control-T47DUVS001-1 (Item K)	1 vial of lyophilized powder from T47D cell lysate.	1 week at -80°C
Pan Detection Antibody P53 (Item C-2)	2 vials of biotinylated anti-pan-P53 (1 vial is enough to assay half microplate).	5 days at 4°C
HRP-Streptavidin concentrate (Item G)	1 vial (200 µl/vial) of 300x concentrated HRP-conjugated Streptavidin.	Do not store and reuse.
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A
Assay Diluent (Item E2)	15 ml of 5X concentrated buffer. For diluting cell lysate samples, detection antibody (Item C-2), and HRP-Streptavidin concentrate.	1 month at 4°C
Cell Lysate Buffer (Item J)	5 ml 2X cell lysis buffer (does not include protease and phosphatase inhibitors).	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

IV. ADDITIONAL MATERIALS REQUIRED

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.

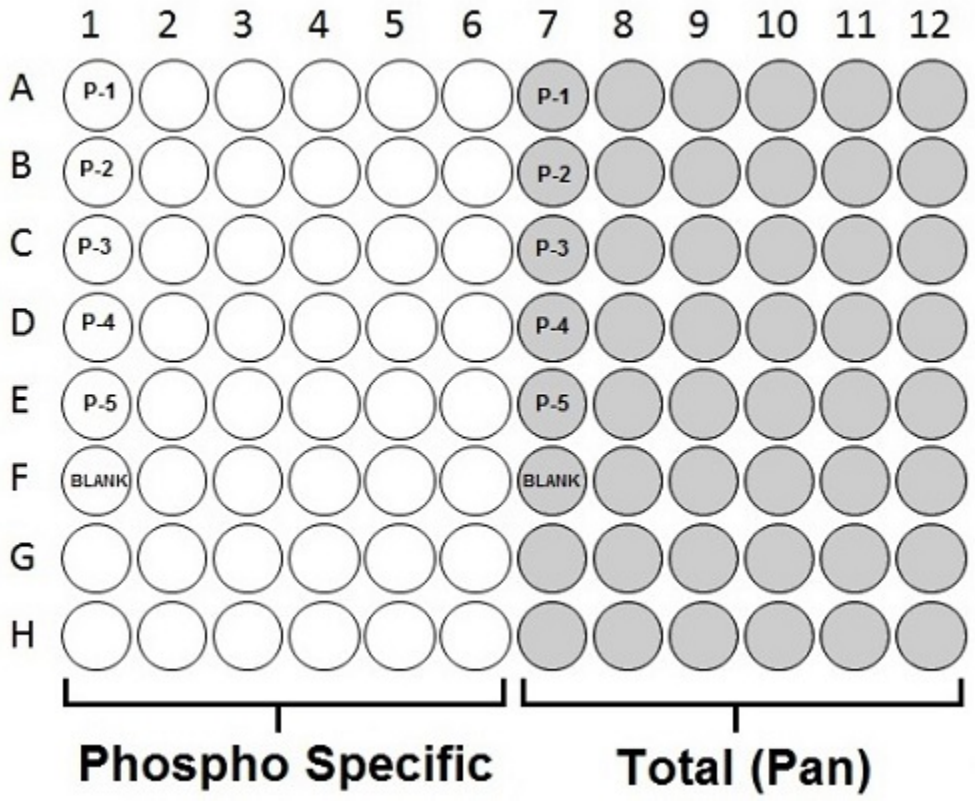
V. SAMPLE PREPARATION

Cell Lysate Preparation: Rinse the cells with PBS, making sure to remove any

remaining PBS before adding the lysis buffer. Solubilize cells at 4 x 10^7 cells/ml in prepared Cell Lysate Buffer (Item J) (see Reagent Preparation step 3). Pipette up and down to resuspend the pellet. 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 supernatantes 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, we recommend a serial dilution, such as a 5-fold to 50-fold dilution, for your cell lysates with prepared Assay Diluent (Item E2) (see Reagent Preparation step 2) 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.



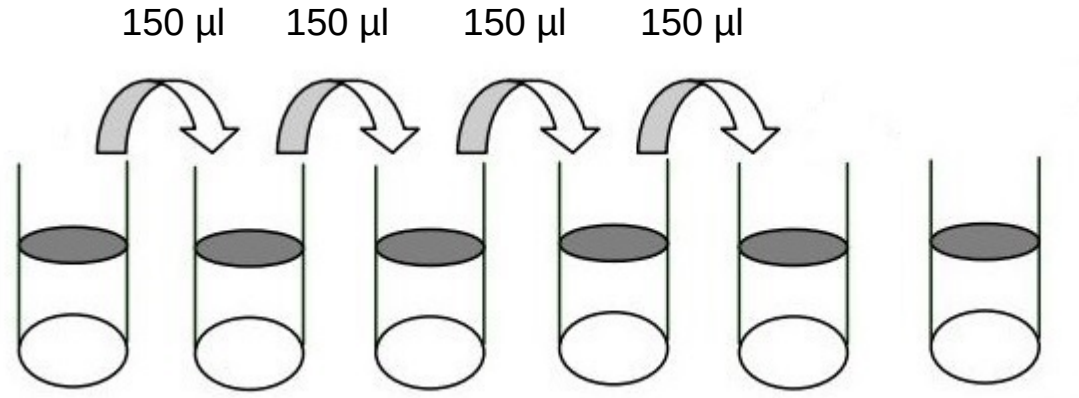
VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25ºC) 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). We also recommend the addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use.

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 centrifuge of the positive control vial, and then pipetting the supernate only for the assay. Pipette 300 µl 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).



	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-pan-P53 antibody: Briefly spin the vial of

biotinylated anti-pan-P53 (Item C-2). Add 100 µl of 1X Assay Diluent into the vial to prepare a pan 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 55-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 step 7 of the Assay Procedure.

For example: Briefly spin the vial. Add 10 µl of HRP-conjugated Streptavidin concentrate into a tube with 3.0 mL 1x Assay Diluent, pipette up and down to mix gently to prepare a 300-fold diluted HRP-conjugated Streptavidin solution. Mix well.

VII. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is strongly recommended to run all positive controls and samples in at least duplicate. It is also recommended to run the positive controls in singlet for each of the pan and phospho-specific antibodies.

2. See plate layout (page 5) and label removable 8-well strips as appropriate for your 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 4ºC 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 multi-channel 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-pan-P53 antibody (see Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

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

7. 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.

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

9. 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.

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

VIII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and positive control as instructed.

2. Add 100 µl positive control or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4ºC with gentle shaking.

3. Add 100 µl prepared detection antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

4. Add 100 µl prepared HRP-Conjugated solution. Incubate for 45 minutes at room temperature with gentle shaking.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

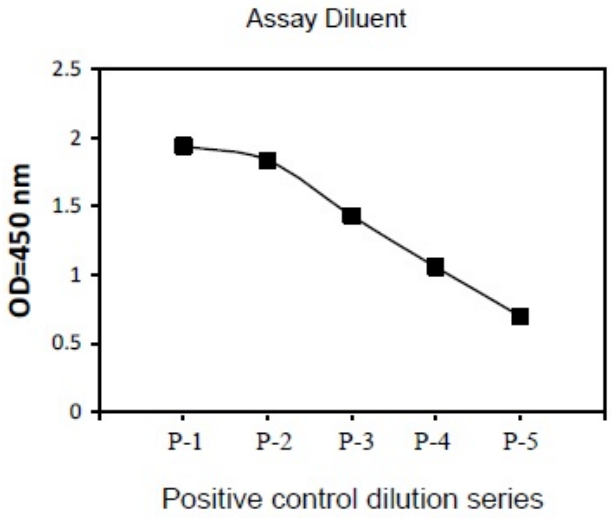
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

IX. TYPICAL DATA

Calculate the mean absorbance for each sample. Then, subtract the average zero (blank) optical density from each sample mean and set of singlet positive controls.

A. Positive Control

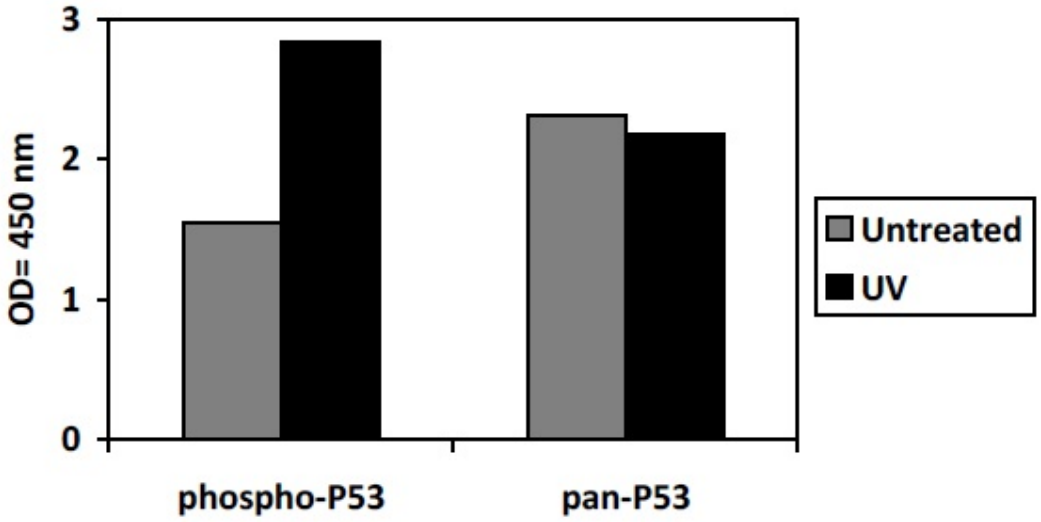
T47D cells were exposed to 50J/m2 of UV light followed by a 4 hours recovery period. Cells were solubilzed at 4 x 10^7 cells/ml in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA (see Reagent Preparation step 4).



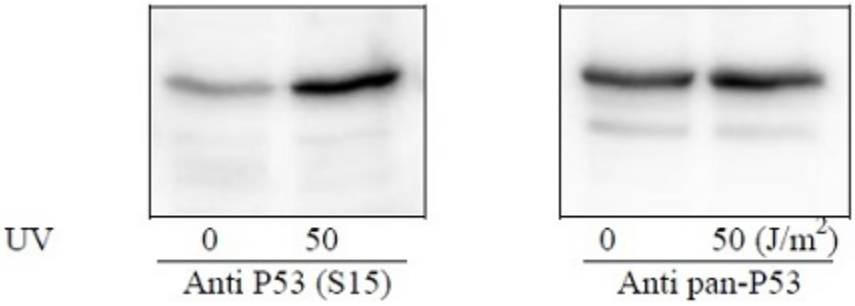
B. UV irradiation of T47D Cell Line

T47D cells were untreated or exposed to 50J/m^2 of UV light followed by a 4 hours recovery period before lysis. Cell lysates were analyzed using this phosphoELISA and Western Blot.

i. ELISA



ii. Western-Blot Analysis



X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Low signal in samples	• Sample concentration is too low • Improper preparation of detection antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Increase sample concentration • Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time; assay procedure step 3 may be done overnight • Check pipettes and ensurecorrect preparation
High signal in samples	• Sample concentratin is too high	• Reduce sample concentration
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution • Improper primary or secondary antibody dilution	• Store your positive control at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate • Ensure correct dilution

RayBio® ELISA Kits

Over 2,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.