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ISO 13485:2016 cGMP

Ubiquitinated p53 Quantitative ELISA Kit

Catalog #: PTE-UbiP53-Q

User Manual
Last Revised: August 21, 2024

Introduction

The RayBio® Ubiquitinated p53 Quantitative ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human, mouse, rat ubiquitinated p53. An anti-pan p53 antibody has been coated onto a 96-well plate. Standard and samples are pipetted into the wells and p53 present in a sample is bound to the wells by the immobilized antibody. The wells are washed, and biotinylated anti-ubiquitin antibody is used to detect ubiquitinated p53. After washing away unbound antibody, HRP-conjugated 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 ubiquitinated p53 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage / Stability

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 prepared reagent storage, see kit contents on the next page.

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Kit Components

Name	Catalog #	Size / Qty	Description	Storage / Stability After Preparation
Anti-Pan-p53 Microplate	PTE-UbiP53-Q-A	96 wells	Microplate coated with anti-pan-p53 antibody.	1 month at -20°C*
Ubiquitinated-p53 Standard	PTE-UbiP53-Q-STD	2 vials	Ubiquitinated p53. 1 vial is sufficient to run each standard in duplicate.	1 week at -20°C
Anti-Ubiquitin Detection Antibody	PTE-Ubi-C	2 vials	Biotinylated anti-ubiquitin antibody. 1 vial is sufficient to assay half the microplate.	5 days at 4°C
HRP-Streptavidin	EL-HRP	200 µl	300X concentrated HRP-conjugated streptavidin.	Do not store and reuse.
Wash Buffer	EL-ITEMB	25 ml	20X concentrated wash buffer	1 month at 4°C
Assay Diluent B	EL-ITEME	15 ml	5X concentrated assay diluent	1 month at 4°C
Lysis Buffer	EL-Lysis	5 ml	2X cell lysate buffer	1 month at 4°C
TMB One-Step Substrate Reagent	EL-TMB	12 ml	3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	N/A
Stop Solution	EL-STOP	8 ml	0.2 M sulfuric acid	N/A

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

Additional Materials Required

- Microplate reader capable of measuring absorbance at 450 nm
- Protease and Phosphatase inhibitors.
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1-liter graduated cylinders
- Log-log graph paper or computer and software for ELISA data analysis.
- Absorbent paper
- Tubes to prepare standard or sample dilutions
- Distilled or deionized water
- Shaker

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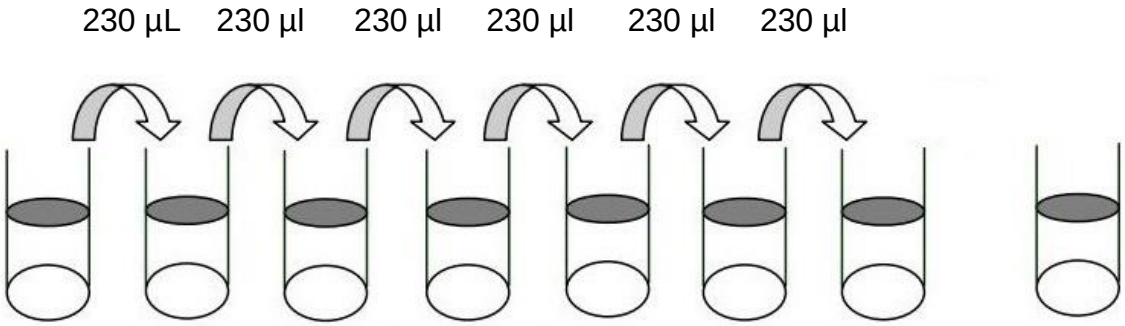
Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.
2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Lysis Buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate). We also recommend the addition of protease inhibitors (not included) to the lysis buffer prior to use.
4. Preparation of Ubiquitinated p53 Standard: Briefly spin the Standard Vial. Add 460 µl of prepared 1X Assay Diluent B to prepare a 20 ng/ml standard solution. Gently mix the powder to allow it to dissolve thoroughly. Pipette 230 µl 1X Assay Diluent B into each tube. Use the standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent B serves as the blank.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Blank
Diluent volume	460 µl	230 µl	230 µl	230 µl	230 µl	230 µl	230 µl	230 µl
Conc.(ng/ml)	20	10	5	2.5	1.25	0.625	0.3125	0

5. If the Wash Buffer concentrate 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 Anti-Ubiquitin Detection Antibody: Briefly spin the vial of biotinylated Anti-Ubiquitin Detection Antibody. Add 100 µl of 1X Assay Diluent B into the vial to prepare a ubiquitin 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

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should then be diluted 55- fold with 1X Assay Diluent B and used in step 4 of the Assay Procedure.

7. Preparation of HRP-Streptavidin: Briefly spin the vial of HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 300-fold with 1X Assay Diluent B and used in step 7 of the Assay Procedure.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended to run all standards and samples in at least duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 4) and 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 auto-washer. 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-Ubiquitin Detection 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 1 hour 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 to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

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Assay Procedure Summary

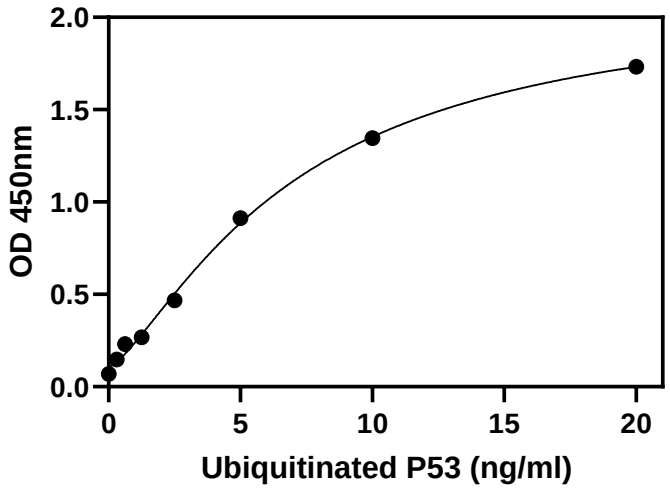
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl of each standard and sample to each well. Incubate 2.5 hours at room temperature or overnight at 4ºC with gentle shaking.
3. Add 100 µl prepared detection antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.
4. Add 100 µl prepared HRP-Streptavidin solution. Incubate for 1 hour 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.

Typical Data

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.



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B. Sensitivity

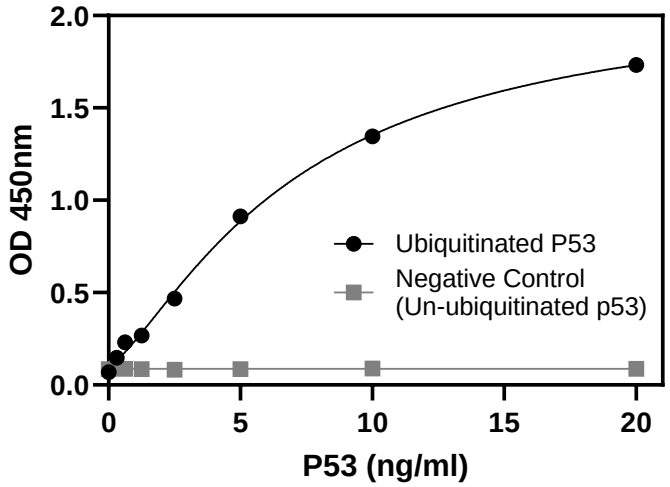
The minimum detectable concentration of Ubiquitinated p53 is 0.07ng/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank.

C. Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

D. Specificity

This ELISA antibody pair detects Human, Mouse, Rat Ubiquitinated p53.



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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 ensure correct preparation
High signal in samples	• Sample concentration 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 washing. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Failure to add stop solution • Improper primary or secondary antibody dilution	• Store your standard 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