



TF-Detect™ Human p53 Activity Assay Kit
For rapid and sensitive detection and quantification of active p53

Cat. No. TAK-P53-196 (1 plate, 96 reactions)

User Manual

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850
USA

301-762-0888
866-360-9531

inquiry@genecopoeia.com

www.genecopoeia.com

© 2010 GeneCopoeia, Inc.

USER MANUAL

TF-Detect™ Human p53 Activity Assay Kit

- I. Introduction and Principle
- II. Kit Components and Storage
- III. Preparation
- IV. Procedure
- V. Limited Use License and Warranty

I. Introduction and Principle

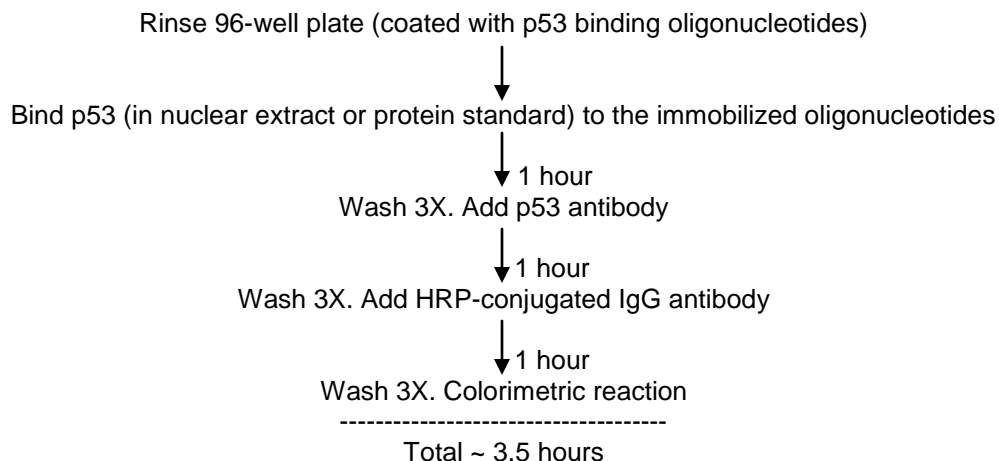
When responding to diverse cellular stresses, tumor suppressor p53 regulates target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. p53 protein binds to a consensus p53-binding site and activates expression of downstream genes that inhibit growth and/or invasion. Mutations in p53 frequently occur in numerous types of human cancers. The p53 mutants lose the tumor suppressor activity¹ by failing to bind to the consensus DNA binding site.

TF-Detect™ Human p53 Activity Assay kit enables fast and sensitive detection and quantification of p53 in a 96-well format². Double-stranded oligonucleotides containing a p53 consensus binding site are immobilized in a 96-well plate. The p53 proteins present in nuclear extracts are captured by the immobilized oligonucleotides specifically and then detected by a p53 antibody and a HRP-conjugated secondary antibody. The colorimetric signal generated by HRP substrate TMB can be easily quantified by spectrophotometry.

A purified recombinant human p53 protein is also provided in the kit for use as protein standard for quantifying and comparing p53 activities of different sample types or time points.

- **Sensitive** – Detects as little as 0.8 ng of human p53 protein per well and performs better than a similar competitor kit (Fig.1).
- **HTS compatible** - Optimized for use on 96-well plate readers for high-throughput screening assays. Single strip (8-well) assay can also be performed.
- **Fast** - 3.5 hours from preparation to detection.
- **Quantitative** – The active and purified human recombinant p53 protein provided in the kit allows users to generate standard curve (Fig.2) and quantify p53 in samples.

Protocol Overview



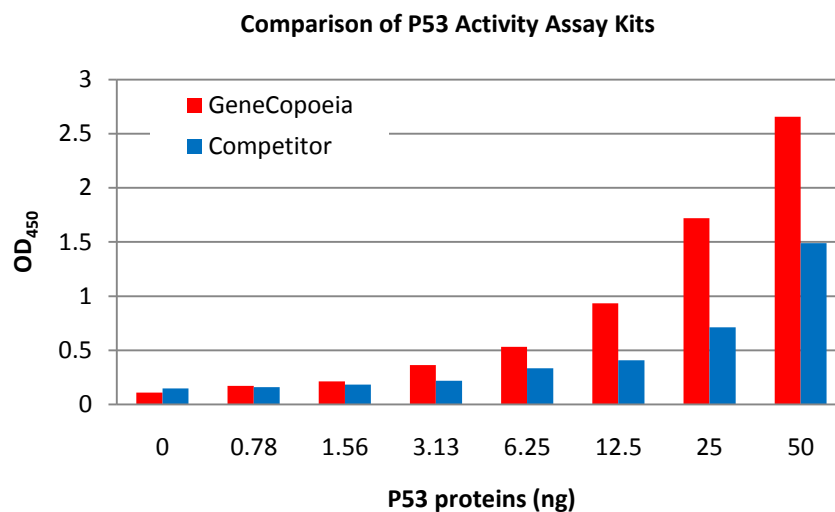


Figure 1. Performance comparison between GeneCopoeia's TF-Detect Human p53 Activity Assay kit and a similar competitor product. A human recombinant p53 protein was detected and quantified using both kits.

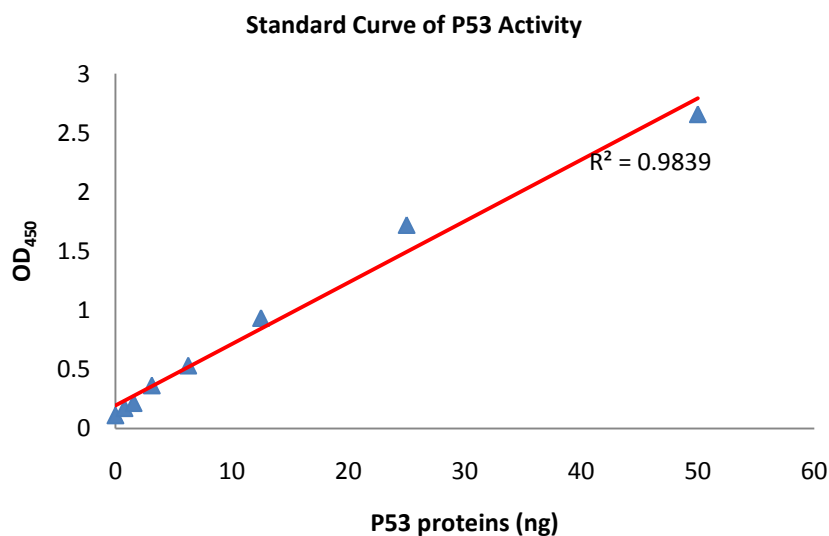


Figure 2. Standard curve for p53 quantitation is generated using GeneCopoeia's purified p53 recombinant protein (included in the kit). The curve is provided for demonstration only.

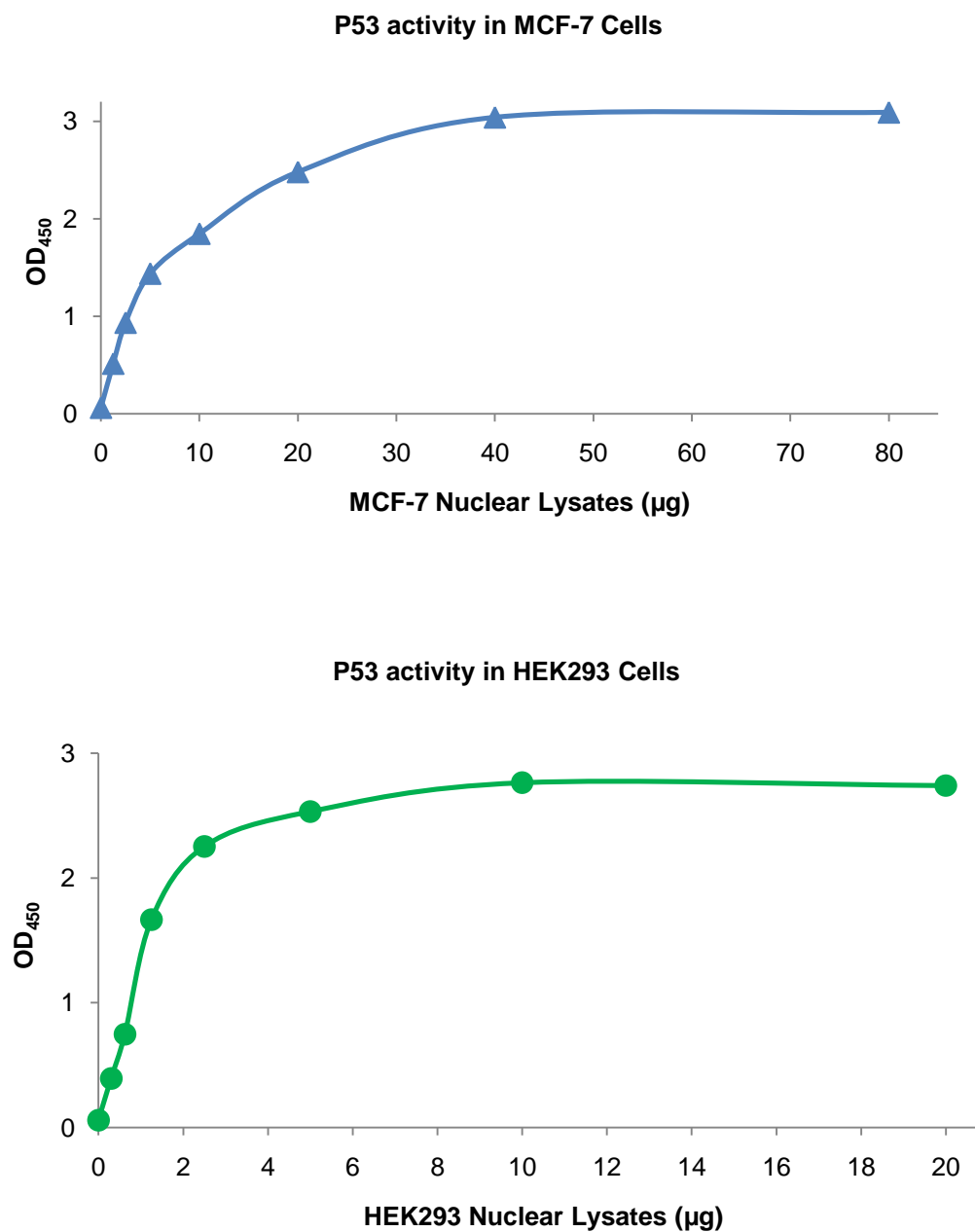


Figure 3. The activity of p53 proteins from the nuclear extracts of MCF-7 (Top) and HEK293 (Bottom) cells were detected using the TF-Detect p53 Activity Assay Kit. Both cell types were treated with 0.2mM H₂O₂ for 3 hours before harvesting. The cell nuclear extracts were prepared following the Preparation of Nuclear Extract protocol in the Appendix.

II. Kit Components and Storage

Cat. No. TAK-P53-196 (1 plate, 96 reactions)

Components	Quantity	Storage temperature
p53 antibody	15 µl	-20°C Stable for at least 6 months
HRP-conjugated IgG	15 µl	-20°C Stable for at least 6 months
Recombinant p53 protein* (0.25µg/µl)	20 µl	-20°C Stable for at least 6 months
Dithiothreitol (DTT) (1M)	100 µl	-20°C Stable for at least 6 months
10x Binding Buffer A	1.5 ml	-20°C Stable for at least 6 months
10x Binding Buffer B	1.5 ml X 2	-20°C Stable for at least 6 months
10x Wash Buffer	25 ml	4°C Stable for at least 6 months
TMB Substrate Solution	12 ml	4°C Stable for at least 6 months
Stop Solution	12 ml	4°C Stable for at least 6 months
96-well p53 assay plate (12 strips)	1 plate	4°C Stable for at least 6 months

*Recombinant p53 can be used as both a positive control and a protein standard.

Materials required but not provided

5 ml and 10 ml graduated pipettes, beakers, flasks, and cylinders

10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips

50 µl to 300 µl adjustable multichannel micropipette, disposable tips, and reservoir

Micro-plate reader capable of reading at 450 nm (620 nm as optional reference wave length)

III. Preparation of reagents

Prepare a little bit extra than required amount to make sure enough buffers are available for experiments. Required amount of reagents per well:

Reagent	1 well
1x Wash buffer	2 ml
1x Binding buffer A (without DTT)*	50 µl
1x Binding buffer A (with DTT at 1:500 dilution)**	50 µl
Sample nuclear extract	10 µl
Recombinant p53 (positive control and protein standard)	10 µl
p53 antibody in 1x Binding buffer B (1:1000)	100 µl
HRP IgG antibody in 1x Binding buffer B (1:1000)	100 µl
TMB substrate	100 µl
Stop solution	100 µl

*Since DTT inhibits the tetramerization of p53 recombinant protein, 1x Binding Buffer A (**without DTT**) is used for the dilution and binding reaction of the recombinant p53.

1x Binding Buffer A (with DTT**) is used for the dilution and binding reaction of sample nuclear extracts.

Wash buffer

Warm up 10x Wash Buffer to room temperature and mix well before use. If crystals have formed in the 10x buffer, warm and mix gently until they have completely dissolved. To prepare 200 ml of 1x Wash Buffer for one 96-well plate assay, add 20 ml of the 10x Wash Buffer into 180 ml distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should be adjusted to 7.8. The 1x Wash Buffer is stable for 30 days at 4°C.

Binding buffer A and B

Warm up 10x Binding Buffer A and B to room temperature and mix well before use. To prepare 100 ml of 1x Binding Buffer, add 10 ml of the 10x buffer to 90 ml distilled or deionized water. Mix gently to avoid foaming. The 1x Binding Buffers are stable for 30 days at 4°C.

For the recombinant p53 as protein standard and positive control, 1x Binding Buffer A **without DTT** should be used, since DTT inhibits the tetramerization of p53 recombinant protein.

For nuclear extract samples, 1x Binding Buffer A **with DTT** should be used. DTT should be added to 1x Binding Buffer A at 1:500.

Samples

We recommend using 10 µl of nuclear extract at 0.2 - 5 µg/µl for each sample well. The total protein amount is 2-50 µg per well. **Note:** The recommended amount is provided as guidance. A broader range of value may be used.

Protein standard curve

For those who wish to quantify the amount of p53 in their samples, GeneCopoeia offers recombinant p53 as a protein standard. Starting with a 250 ng/µl working stock of recombinant p53, make serial dilution in a range of 5 ng/µl to 0 ng/µl using Binding Buffer A (**without DTT**). **Note:** the recommended dilution range is provided as guidance, a broader range of values may be used.

	Recombinant p53 solution	Binding Buffer A (without DTT)	p53 standard concentration	Final volume
A	1 µl of p53 stock (250 ng/µl)	49 µl	5 ng/µl	50 µl
B	25 µl of A	25 µl	2.5 ng/ µl	50 µl
C	25 µl of B	25 µl	1.25 ng/µl	50 µl
D	25 µl of C	25 µl	0.625 ng/µl	50 µl
E	25 µl of D	25 µl	0.312 ng/µl	50 µl
F	25 µl of E	25 µl	0.156 ng/µl	50 µl
G	25 µl of F	25 µl	0.078 ng/µl	50 µl
H	0	60 µl	0 ng/ µl (blank)	60 µl

10 µl of each protein standard concentration will be used to generate the standard curve. The range of protein amount for the standard curve is 0 ng to 50 ng.

Positive control

If quantitation is not necessary, 10 µl of the 2.5 ng/µl p53 protein standard can be used as the positive control.

Primary p53 antibody

Calculate the amount of primary p53 antibody needed to perform the desired experiments and make 1:1000 dilutions with 1x Binding Buffer B (100 µl/well).

Secondary HRP conjugated antibody

Calculate the amount of HRP conjugated antibody needed to perform the desired experiments and make 1:1000 dilution with 1x Binding Buffer B (100 µl/well).

TMB substrate solution

Take appropriate volume of TMB Substrate (100 µl/well) and warm it up to room temperature 1 hour before use. **Note:** Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation. The TMB Substrate Solution may develop a yellow tinge over time. This does not affect the product performance. A blue color in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. After use, discard remaining TMB substrate solution.

Stop solution

Prior to use, take appropriate volume of Stop Solution (100 µl/well). After use, discard remaining Stop Solution.

IV PROCEDURE

Determine the number of wells and strips needed. Store the unused strips at 4°C.

1. Mix all reagents thoroughly yet gently to avoid foaming before use.
2. Rinse the 96-well plate with approximately 200 µl Wash Buffer per well. Empty and tap the plate on absorbent pad or paper towel to remove excess buffer. Be careful not to scratch the surface of the 96-well plate.

Note: Use the plate immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes.

3. Sample wells: Add 50 µl of Binding Buffer A (**with DTT**) to the wells, and then add 10 µl samples (2-50 µg of proteins). Mix well. Duplicated wells for each sample are recommended.

Standard curve wells: Prepare p53 protein standard according to the instruction in Preparation of reagent. Add 10 µl of p53 standard in duplicate to the standard wells and mix with 50 µl of Binding Buffer A (**without DTT**).

Positive control wells (if protein standard curve is not performed): Add 10 µl of 2.5 ng/µl p53 recombinant protein per well. Add 50 µl of Binding Buffer A (**without DTT**) and mix well.

Blank wells: Add 60 µl of Binding Buffer A (without DTT).

4. Cover the plate with a plate cover. Incubate at room temperature for 1 hour by gently rocking the plate. Remove the plate cover and empty the wells.

Wash the plate by gently rocking it for one minute using 200 µl Wash Buffer per well. Repeat three times.

5. Add 100 µl of diluted p53 primary antibody to each well, including the blank wells. Cover the plate with a plate cover. Incubate at room temperature for 1 hour by gently rocking the plate. Remove the plate cover and empty the wells.

Wash the plate by gently rocking it for one minute using 200 µl Wash Buffer per well. Repeat three times.

6. Add 100 µl of diluted HRP conjugated antibody to each well, including the blank wells. Cover the plate with a plate cover. Incubate at room temperature for 1 hour by gently rocking the plate. Remove the plate cover and empty the wells.

Wash the plate by gently rocking it for one minute using 200 µl Wash Buffer per well. Repeat three times.

7. Add 100 µl TMB Substrate Solution to each well (including the blank wells) and mix well. Incubate the plate at room temperature for about 5-15 minutes.

8. Add 100 µl Stop Solution to each well (including the blank wells) and mix well. Read the plate within 5 minutes using a microwell plate reader at 450nm.

VI Reference

1. Hollstein M., et al (1999) Mutat. Res. 431:199-209.
2. Renard P., et al (2001) Nucleic Acids Res. 29: (4) e21.

VII Appendix

Preparation of Nuclear Extract

1. Aspirate medium from a 10 cm plate and wash the cells with ice-cold PBS.
2. Add 1 ml of ice-cold PBS (per 10 cm plate, 4 - 8 x 10⁶ cells). Scrape the cells into PBS and then transfer them into a pre-chilled eppendorf tube.
3. Spin at 4°C, 2,000 rpm for 5 minutes. Discard the supernatant.
4. Resuspend the cell pellet in 200 µl of **Buffer 1** (per 10cm plate, scale up or down proportionally for other size culture vessels). Incubate on ice for 15 minutes to allow cells to swell.
5. Add 20 µl of **Buffer 2** to the cells. Vortex for 10 seconds. Then incubate on ice for 5 minutes.
6. Spin at 4°C, 14,000 rpm for 3 minutes.
7. Transfer the supernatant (cytoplasmic proteins) into a new eppendorf tube.
8. Add another 200 µl of **Buffer 1** to the cell pellet and mix gently.
9. Spin at 4°C, 14,000 rpm for 3 minutes.
10. Transfer and combine the supernatant in the cytoplasmic protein tube from step 7.

11. Resuspend the pellet with 200 µl of ice-cold **Buffer 3**. Vortex for 30 seconds. (Optional: Sonicate for 2-3 seconds to break down the pellet.) Then rotate vigorously at 4°C for 30 minutes.
12. Spin at 4°C, 14,000 rpm for 10 minutes.
13. Transfer the supernatant (nuclear proteins) into a fresh, pre-chilled tube. Measure the protein concentrations. Leave on ice if use immediately, or store aliquots at -80°C until use.

Buffer 1: 25 mM HEPES, pH 7.4
10 mM KCl
1.5 mM MgCl₂
1 mM DTT
100 mM PMSF

Buffer 2: 10% IGEPAL

Buffer 3: 25 mM HEPES, pH 7.4
420 mM NaCl
25% Glycerol
1.5 mM MgCl₂
0.2 mM EDTA
1 mM PMSF

VIII Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of TF-Detect Human p53 Activity Assay Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

© 2010, GeneCopoeia, Inc.