PRODUCT INFORMATION & ELISA MANUAL

p53 Antibody Pair [HRP]

NBP2-79658

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked Immunosorbent Assay for quantitative detection of Cynomolgus Monkey p53.

For research use only.
Not for diagnostic or therapeutic procedures.

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BACKGROUND

p53, also known as Tp53, is a DNA-binding protein which belongs to the p53 family. It contains transcription activation, DNA-binding, and oligomerization domains. p53 protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. p53 (Tp53) is a transcription factor whose protein levels and post-translational modification state alter in response to cellular stress (such as DNA damage, hypoxia, spindle damage). Activation of p53 begins through a number of mechanisms including phosphorylation by ATM, ATR, Chk1 and MAPKs. MDM2 is a ubiquitn ligase that binds p53 and targets p53 for proteasomal degradation. Phosphorylation, p14ARF and USP7 prevent MDM2-p53 interactions, leading to an increase in stable p53 tetramers in the cytoplasm. Further modifications such as methylation and acetylation lead to an increase in Tp53 binding to gene specific response elements. Tp53 regulates a large number of genes (>100 genes) that control a number of key tumor suppressing functions such as cell cycle arrest, DNA repair, senescence and apoptosis. Whilst the activation of p53 often leads to apoptosis, p53 inactivation facilitates tumor progression. It is postulated to bind to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mutants of p53 that frequently occur in a number of different human cancers fail to bind the consensus DNA binding site, and hence cause the loss of tumor suppressor activity. Defects in Tp53 are a cause of esophagealcancer, Li-Fraumeni syndrome, lung cancer and adrenocortical carcinoma.

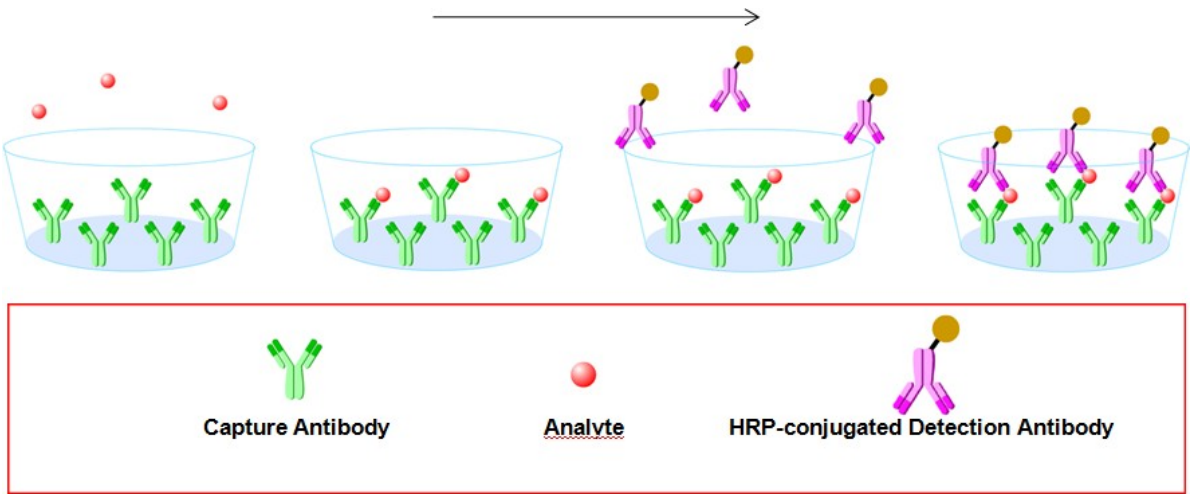
PRINCIPLE OF THE TEST

The Novus Biologicals p53 Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Cynomolgus p53 coated on a 96-well plate. Standards and samples are added to the wells, and any Cynomolgus p53 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-Cynomolgus p53 monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Cynomolgus p53 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- The Cynomolgus p53 Antibody Pair [HRP] is for the quantitative determination of Cynomolgus p53.
- This p53 Antibody Pair [HRP] contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 1 mg/mL of mouse anti-Cynomolgus p53 monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

Detection Antibody – 0.2 mg/mL of mouse anti-Cynomolgus p53 monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4℃). Dilute to working concentration of 1 µg/mL in detection antibody dilution buffer before use.

Standard – Each vial contains 160 ng of recombinant Cynomolgus p53. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 10000 pg/mL is recommended.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2 µm filtered

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4℃ and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100μL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

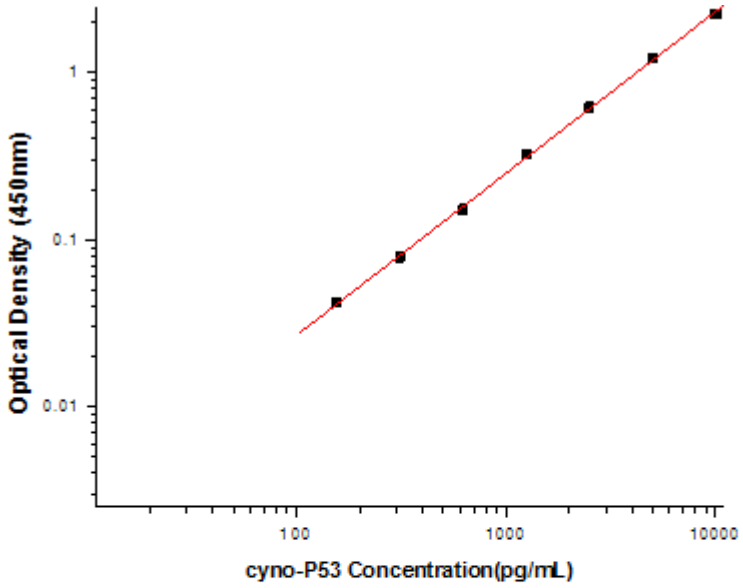
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 μL of substrate solution to each well. Incubate for 20 minutes at room temperature (**if substrate solution is not as requested, the incubation time should be optimized**). Avoid placing the plate in direct light.
6. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/mL)	Zero standard subtracted OD
0	0
156.25	0.042
312.5	0.079
625	0.151
1250	0.324
2500	0.622
5000	1.225
10000	2.219

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Cynomolgus p53 was determined to be approximately **156.25 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Notes