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Not for use in diagnostic procedures.

p53 pan ELISA

Version 06
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Cat. No. 11 828 789 001
96 test

One-step -immunoassay

Photometric one-step-enzyme-immunoassay for the quantification of p53 (human, mouse, rat) in cell homogenates, plasma or serum

Store at +2 to +8°C

sigma-aldrich.com

Contents

Kit contents

1. **Anti-human-p53 pan-peroxidase** (POD), polyclonal antibody from sheep, lyophilizate (bottle 1, red cap)
2. **Human p53 standards,** six concentrations between 0 pg/ml and 1200 pg/ml (see lot-specific label), lyophilizates (bottle 2a–f, orange caps)
3. **Incubation buffer/Sample diluent,** 50 ml, ready-to-use solution (bottle 3, white cap)
4. **10 × washing buffer,** 100 ml (bottle 4, blue cap)
5. **TMB substrate solution,** 26 ml ready-to-use solution (bottle 5)
6. **TMB stop solution,** 8 ml ready-to-use (bottle 6)
7. **Streptavidin coated microtiter plate,** precoated with anti-p53-biotin, monoclonal from mouse, 8-well modules in a frame, 96 wells total
8. **Self adhesive plate cover foils,** 2 foils
9. **Pack insert** (booklet)

Stability

The kit is stable at +2 to +8°C until expiry date (see lot-specific lable imprint). For stability and storage conditions of working solutions see chapter 6. Microtiter plate strips, that have not been used, can be kept at least for 4 weeks if stored in the dark and tightly closed at +2 to +8°C.

1. Introduction

The p53 tumor suppressor is involved in the control of cell growth and programmed cell death. Inactivation of p53 by mutation, deletion or certain viral and cellular proteins increases the susceptibility to malignant transformation. Wild-type p53 levels in normal cells and tissues were found to be very low whereas in mammalian tumors and tumor cell lines mutant p53 polypeptide is often detectable in high concentrations. This accumulation of mutant p53 protein is the result of a conformational change in the protein, with consequent prolonged half-life and stability. Mutations in the p53 gene are among the most common genetic alterations in human malignancies.

2. Application

Research kit for the quantfication of p53 (human, mouse, rat) in serum, plasma or homogenates from tumor-tissue or tumor cell lines. This assay is intended for use as a research tool in further understanding the meaning of p53 levels in plasma, serum and cell homogenates.

3. Test principle (one-step-immunoassay)

The assay is based on a quantitative “sandwich ELISA” principle. The biotin-labeled capture antibody is pre-bound to the streptavidin-coated microtiter plate. During one single incubation step the p53-containing sample (specimen or standard) reacts with capture antibody and peroxidase-labeled detection antibody to form a stable immuno-complex. Subsequent to the washing step, the peroxidase bound in the complex is developed by tetramethylbenzidine (TMB) as a substrate. The photometrically determined color is proportional to the concentration of p53.

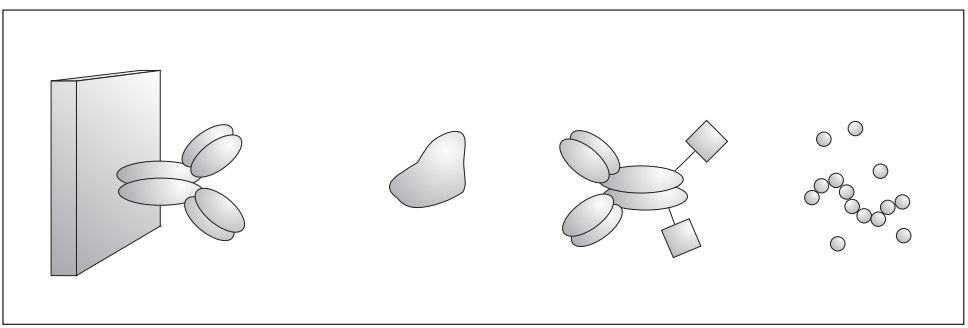

Fig.1: Test principle

4. Assay characteristics

4.1 Sensitivity

The lower limit of detection (LLD) of the assay, which is commonly used to express analytical sensitivity has been determined from four independent experiments. In this study the mean signal of zero-standard plus two standard deviations was calculated from the standard curve to be 9 pg/ml.

A p53 concentration of 50 pg/ml will result in a signal of approximately two-times the signal of the zero-standard.

4.2 Measuring range

The linear measuring range of the ELISA is between 50 pg/ml and 1000 pg/ml

4.3 Specificity

The biotin-labeled capture antibody from mouse recognizes a conserved, pantropic, denaturation stable antigenic determinant of the p53 protein (human, mouse, rat). The peroxidase-labeled detection antibody is highly specific for wild-type and mutant p53 from different species.

4.4 Precision

4.4.1 Intra-assay variance To determine the intra assay variance, three different concentrations of p53 were added to each of three different sera. The resulting p53 concentrations were then determined in 5-fold measurements. A variance of $\leq$ 4.5% was established.

4.4.2 Inter-assay variance To determine the inter assay variance, three different concentrations of p53 were added to each of three different sera. The resulting p53 concentrations were then determined in 5-fold measurements on four successive days. In this experiment a variance of $<$ 12.5% was established.

4.4.3 Recovery For the evaluation of the recovery, each of three different sera were spiked with three different known concentrations of p53 and one serum sample was left untreated. The concentrations in the spiked and untreated samples were measured in 5-fold determinations. The recovery is defined as the difference between the measurements of the spiked and unspiked samples. In serum, a recovery of 80–115 % of the added p53 was found over the the whole measurement range.

4.5 Human p53 standards

The p53 standards (mutant) derived from the human epidermoid carcinoma cell line A431 (ATCC CRL 1555) is lyophilized in an artificial stabilizing matrix.

4.6 Sample material

Tissue homogenates, cell lysates, serum, plasma may be used as sample.

4.7 Assay time

2.5 hours at +15 to $\leqslant$25°C.

4.8 Warnings/Precautions

- Use always the same assay procedure to minimize inter-assay variances.
- Do not substitute kit reagents with those from other lots.
- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light during storage or incubation time.
- Avoid contact of kit reagents with oxidizing agents and metal.
- Exposure to sodium azide will inactivate the conjugate.
- Avoid contact of skin with kit reagents.
- If the substrate stop solution (1 M sulfuric acid) comes into contact with skin or eyes, wash immediately with sufficient amounts of water and contact a physician.
- Strictly perform the assay at the recommended incubation times and temperature.
- Use plastic disposables, avoid glass ware.
- Use polypropylene test tubes for sample dilutions.

4.9 Storage instructions

Store kit reagents, if not otherwise stated, at +2 to +8°C when not in use. Bring kit reagents to +15 to +25°C before starting the assay.

5. Sample preparation

Sample material must not contain azide!

5.1 Serum

Use a serum separator or clot tube and allow samples to coagulate at +15 to +25°C. Avoid hemolysis (see below). Within 30 min. after coagulation, spin samples at 700× *g* for 15 min at +15 to +25°C and collect serum. Aliquot your samples and store at −15 to −25°C. Use sample undiluted (100 µl) or diluted with sample diluent (*e.g.* 1:5).

5.2 Plasma

Collect blood using EDTA or citrate as anticoagulantia. After separation of plasma, collect it rapidly by centrifugation of your samples at 700× g for 15 min at +15 to +25°C. Aliquot your samples and store at −15 to −25°C. Plasma samples may be used undiluted (100 µl) or diluted with sample diluent (*e.g.* 1:5).

5.3 Cell lysates/tissue homogenates

Prepare extracts by detergent lysis using low-salt RIPA buffer (*e.g.* 20 mM Tris, 0.5 mM EDTA, 1.0% Nonidet P40, 0.5% sodium deoxycholate, 0.05% SDS, 1 mM PMSF, 1 µg/ml Aprotinin, 2 µg/ml Leupeptin). Place sample on ice. Add 1–5 volumes of RIPA buffer (tissue) or 1 ml per 10^7 cells. Use a Dounce homogenizer or any other type of micro-homogenizer for desintegration (only tissue). Centrifuge at 10 000× *g* for 10 min. Collect supernatant and dilute an appropriate amount by 1:5 (at minimum) with sample diluent (*e.g.* 50 µl plus 200 µl).

6. Preparation of working solutions (15–25°C).

Solution 1: Anti-p53-POD (bottle 1):
Reconstitute the lyophilizate in 1 ml of redist. water for 10 min and mix thoroughly. The reconstituted solution is stable for at least 3 months at +2 to +8°C.

Solutions 2a–2f: p53 Standards (bottle 2a - 2f):
Reconstitute the lyophilizates in 500 µl of redist. water for 10 min and mix thoroughly. Keep the portion required for the test series at +2 to +8°C. Aliquot the rest to be used for subsequent standard curves and store this at −15 to −25°C.

Solution 3: Incubation buffer/sample diluent (bottle 3):
Ready-to-use, stable at +2 to +8°C.

Solution 4: Washing buffer, 10× (bottle 4):
Dilute content of the bottle 1:10 with redist. water (100 ml plus 900 ml).
Stable at +2 to +8°C.

Solution 5: TMB substrate solution (bottle 5):
The substrate solution is ready-to-use. Fill the required quantity in a suitable vial immediately before use. The solution reacts to light if exposed over a longer period. Do not pipette directly from the bottle into the wells. The solution is stable until the expiry date of the pack (see lot-specific label imprint) if stored at +2 to +8°C.

Solution 6: TMB stop solution (bottle 6):
Ready-to-use, stable at +15 to +25°C.

Solution 7: Anti-p53-POD, working dilution (1:15):
Do only prepare the amount of immunoreagent you need for the day. For example, to prepare 10.5 ml of immunoreagent, add 700 µl anti-p53-POD (solution 1) to 9.8 ml of incubation buffer (solution 3) and mix. This amount is sufficient for 96 wells.

7. Assay procedure

7.1 General recommendations

Important: All reagents should be equilibrated at +15 to +25°C before starting the test. Only the microtiter plate included must be used for the assay.

A separate calibration curve has to be performed for each individual test series. All measurements should be carried out in duplicate.

Sample and standard solutions must be pipetted very carefully.

The test is designed for use in combination with a plate shaker. If a suitable shaker is not available, the test can still be carried out under the described conditions. However, signal levels may vary to a certain extent, which will influence the test precision negatively.

The substrate reaction can be carried out without using stop solution and then measured at 370 nm (measuring wavelength) against 492 nm (reference wavelength). However, the absorbance values will be 25–30% lower.

Reagents of different lots must not be used in one test series.

7.2 Pipetting scheme for the microtiter plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl	P2	P2								
B	Sa	Sa										
C	Sb	Sb										
D	Sc	Sc										
E	Sd	Sd										
F	Se	Se										
G	Sf	Sf										
H	P1	P1									P41	P41

Legend:
Bl = blank (= incubation buffer)
Sa–Sf = standards 2a–2f
P1–41 = sample 1–41

7.3 Working instructions

7.3.1 Pipetting of standards/ samples and antibody

Pipette first 100 µl of standards (solutions 2a–2f) or samples (for dilution see under sample preparation, chapter 5.) very carefully into the wells. Then add 100 µl of anti-p53-POD (solution 7) to all wells supposedly containing standard/samples. Cover the microtiter plate tightly with the included adhesive cover foil and incubate for 2 hours at +15 to +25°C on a shaker. A standard curve has to be determined individually for each experiment.

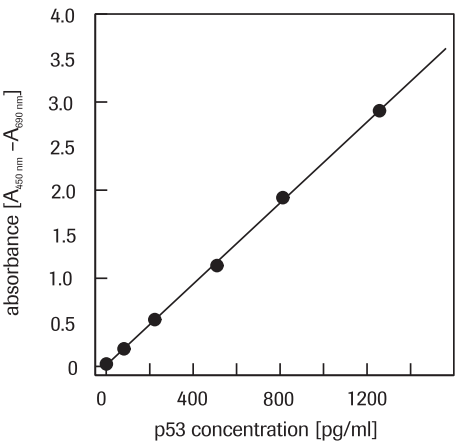

Fig.2: Typical standard curve, used for the calibration of prediluted serum samples

Note: Absorbance values depend very much on the substrate incubation time and the micotiter plate reader system. Therefore, the a. m. absolute values must not be taken as a guideline.

7.3.2 Washing

Remove incubation buffer thoroughly by tapping off or suction. Rinse wells five times with 300 µl washing buffer (solution 4) and remove washing solution carefully.

7.3.3 Substrate reaction

Pipette 200 µl of substrate solution (solution 5) into the wells. Cover the microtiter plate tightly with the adhesive cover foil included and incubate at room temperature on a shaker at 300 rpm until colour development is sufficient for photometric detection (10–20 min).

Protection from light will reduce the background level of the substrate!

7.3.4 Stop reaction

Pipette 50 µl of stop solution (solution 6) into each well. Incubate the plate for approx. 1 min on the shaker at 300 rpm (or mix thoroughly).

7.3.5 Instruments and Measurements

Assay results are quantitated spectrophotometrically at 450 nm using a microtiter plate reader (reference wavelength: 690 nm) against blank. Measuring should be carried out within 5 min after adding stop solution.

Alternative procedure without stop solution: Measure at 370 nm (reference wavelength: 492 nm) against blank. Maximal absorbance reading for standard 2f should be 2.5.

7.3.6 Interpretation

Average the values from the double absorbance readings from standards/samples. The calibration curve is constructed by plotting the average absorbance values of standards (y-axis) versus the standard concentrations (x-axis), or by processing the data with an appropriate laboratory data system. Sample concentrations can then be determined from the calibration curve. Samples with values exceeding the measurement range should be diluted further and run again. In this case, the additional dilution factor has to be taken into account when calculating the amount.

7.4 Changes to Previous Version

Editorial changes.

7.5 Trademarks

7.6 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

7.7 Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

8. Ordering Information

Kits

Product	Pack size	Cat. No.
hIL-1β-ELISA	1 kit (96 tests)	11 600 729 001
hIL-4 ELISA	1 kit (96 tests)	11 921 002 001
hIL-6-ELISA	1 kit (96 tests)	11 534 475 001
hIL-6 Receptor (p80)-Ligand-Binding-Assay	1 kit (96 tests)	11 746 391 001
hIL-10-ELISA	1 kit (96 tests)	11 699 342 001
EPO-ELISA	1 kit (96 tests)	11 693 417 001
MIA ELISA	1 kit (96 tests)	11 976 826 001
hTNF-α-ELISA	1 kit (96 tests)	11 425 943 001

Antibodies

Anti p53 protein, pan	200 µg	11 810 928 001

Parts of some of these products have been prepared from blood of donors tested individually by FDA approved test and found to be free from HBsAg and antibodies to HIV (human immuno-deficiency virus).

9. Quick reference protocols

9.1 Required solutions (sufficient for 96 tests)

Solution	Content	Used for
1	**Anti-p53-POD** (bottle 1): dissolve in 1 ml redist. water	solution 7
7	**Anti-p53-POD working dilution:** for 10.5 ml of reagent add 700 µl of solution 1 to 9.8 ml of solution 3 and mix thoroughly	step 1
2a–2f	**p53 standards** (bottle 2a–2f): dissolve in 500 µl redist. water	step 1
3	**Incubation buffer/sample diluent** (bottle 3): ready-to-use solution	samples and solution 7
4	**Washing buffer** (bottle 4): dilute 1:10 with redist. water	step 2
5	**TMB substrate solution** (bottle 5): ready-to-use solution	step 3
6	**Stop reagent** (bottle 6): ready -to-use	step 4b

9.2 Working procedure flow sheet

Steps	Procedure	Volume/well	Time/ Temperature
1	Pipette first 100 µl of **solution 2a–2f** or **(un-)diluted sample** respectively into MP wells according to the pipetting scheme Pipette 100 µl of **solution 7** into MP well	100 µl 100 µl	 incubate for 2 h at RT 300 rpm on a shaker
2	Wash five times with **solution 4**	5× 300 µl	5× 1 min
3	Add 200 µl of **solution 5** to MP wells	200 µl	approx. 10–20 min at RT 300 rpm on a shaker
4a	Alternative without stop reagent: measure absorption at 370 nm (reference wave length: 492 nm)		
4b	Alternative with stop reagent: add 50 µl of solution 6 to MP well and subsequently measure absorption at 450 nm (reference wavelength 690 nm)	50 µl	approx. 1 min at RT 300 rpm on a shaker

MP = microtiter plate
RT = at 15–25°C

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