



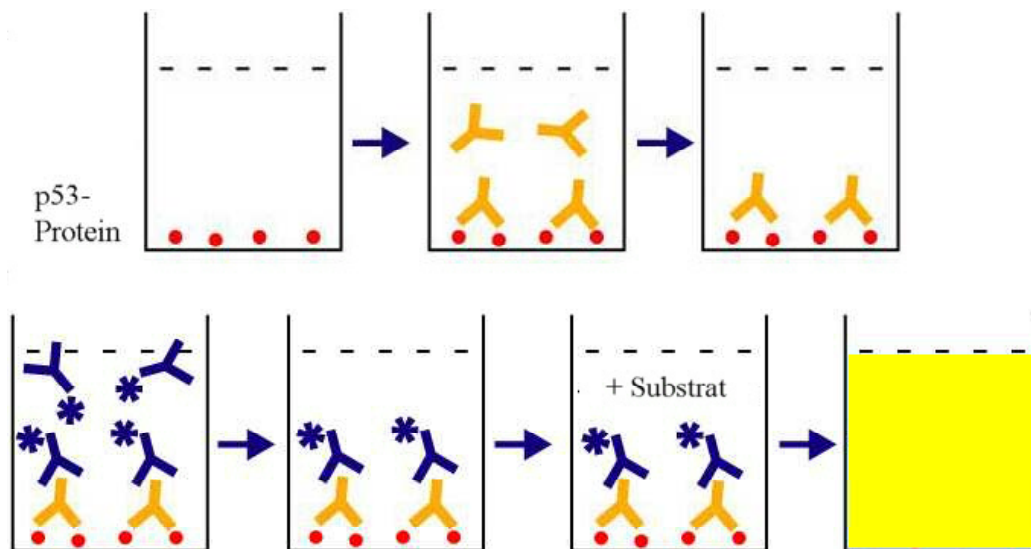
STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE

# p53-autoantibodies ELISA<sup>plus</sup>

Cat. No. p53 Aab

## Product description

Version:015-17.03.2011













**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## Contents

	page
<b>1 Introduction</b>	<b>3</b>
<b>2 Testing Principle</b>	<b>5</b>
<b>3 Warnings and recommendations</b>	<b>5</b>
<b>4 Equipment not provided</b>	<b>5</b>
<b>5 Reagents provided</b>	<b>5</b>
5.1 Risk and safety statements	6
<b>6 Storage and expiry</b>	<b>7</b>
6.1 Waste disposal	7
<b>7 Sample collection and preparation</b>	<b>7</b>
<b>8 Assay procedure</b>	<b>8</b>
8.1 Reagent preparation	8
8.2 Working procedure	8
<b>9 Analysis</b>	<b>9</b>
9.1 Allowed absorption range of controls	9
9.2 Internal Quality Assurance	9
<b>10 Interpretation of results</b>	<b>9</b>
10.1 Example	10
<b>11 Trouble shooting</b>	<b>12</b>
<b>12 Test-characteristics</b>	<b>13</b>
<b>13 Literature</b>	<b>14</b>
<b>14 Assay summary</b>	<b>16</b>

Symbol	Explanation
	Lot Number
	Article Number
	Expiry.....
	Storage Temperature

Symbol	Explanation
	Notice product description
	Reagent for in-vitro diagnostic
	Manufacturer
	CE symbol in accordance to 98/79/EG



STZ Ang. Biol. Chem., Schulzenstr.4, D-68259 Mannheim, Germany



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **1 Introduction**

The p53 gene is altered in up to 60 % of most types of human cancer arising from a wide spectrum of tissues. This leads to abnormal p53 protein accumulation inside the cancer cell. Some patients with an abnormal p53 function develop in a yet unclear mechanism an immunresponse against p53 protein. p53 autoantibodies gain increasing attention since several studies (1-14) described their detection in sera from patients with various malignancies (e.g., colorectal, ovarian, oral, head&neck, trophoblastic and lung carcinomas). The presence of p53 autoantibodies seems to be associated with more progressive cancers and reduced disease-free survival of (surgically) treated patients. There is accumulating evidence that p53 autoantibodies may be indicative for a poor prognosis and a higher risk of tumor relapse. p53 autoantibodies have a useful potential in patient monitoring during therapeutical follow-up. A postoperative significant drop in p53 autoantibodies may be the result of complete tumor resection and successful (adjuvant) chemotherapy.

The percentage of positive sera for cancer patients varied over a wide range, which might be due to different diagnostic accuracy of several tests described by Rohayem et al. in (15). Among three tests used, the STZ ELISA (STZ für Angewandte Biologische Chemie) showed the highest diagnostic accuracy with a significant difference from other tests (15).

In early stages of a progressive malignancy like **colorectal cancer** established tumor markers (for example, CEA, carcinoembryonic antigen) are of low sensitivity. Detection of CEA is not linked to the p53 immune response, so p53 autoantibodies evaluation supplies the clinician with additional data. The combination of p53 autoantibodies and tumor marker CEA significantly increases sensitivity of monitoring colorectal cancer patients (16). Due to high specificity (100%), the monitoring of p53 autoantibodies after surgery and adjuvant chemotherapy of colorectal cancer patients has potential for early diagnosis of tumor relapse (1). Likewise, Takeda et al. (12) noticed a significant correlation between curability of colorectal cancer by surgical tumor resection and postoperative disappearance of p53 autoantibodies in these patients.

Zalcman and co-workers (11) could demonstrate that a rapid, specific decrease of the p53 autoantibodies titer was correlated with successful chemotherapy of **lung cancer** patients.

Detection of p53 autoantibodies has a high potential for the differential diagnosis of **gestational trophoblastic tumors** (GTTs) and their serial measurements are clinically useful to monitor disease progression and to assess response to therapy in GTTs (2).

In some malignancies like pancreatic and prostatic carcinomas, leukemias or melanoma, p53 autoantibodies are less frequent and of minor clinical value (3,5).

It must be stressed that p53 autoantibodies are not specific for any particular cancer. However, as these antibodies are only rarely found in patients with non-malignant disorders (see below) or healthy blood donors, their specificity is higher than that of many other tumor markers.



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

Rare exceptions of p53 autoantibodies in benign diseases are patients with autoimmune diseases like systemic lupus erythematosus, Wegener's granulomatosis, and Graves' disease (16). Although the antibody prevalence was comparable to that in various cancers, differences were obvious with respect to the magnitude of the antibody titer. In a quantitative study all p53 autoantibodies positive autoimmune patients had low titers (critical area) close to the cut-off level of the assay (17). This remarkable difference in magnitude underlines the necessity of p53 autoantibodies quantification over a mere qualitative determination. It should be pointed out that whenever p53 autoantibodies are detected, the possibility for a clinically non-apparent cancerous disease must always be taken into consideration.

**One main objective for p53 autoantibodies evaluations are conformation of clinical data and supplementary information in the follow-up of cancer patients.**

The STZ p53 autoantibodies ELISA shows a high signal to noise ratio. Rohayem et al. evaluated the potential of three commercial p53 autoantibodies ELISAs to provide correct diagnostic classification by use of ROC curve analysis using sera of 72 patients with suspicion of malignancy and sera of 72 healthy blood donors as control. Among the three tests used, the solid-phase ELISA (STZ) showed the highest diagnostic accuracy (AUC = 0.902) with a significant difference from other tests (15).

STZ offers an assay, which enables easy quantitative determination of p53 autoantibodies. Therefore you are able to monitor patient specific levels of p53 antibodies during the course of the disease or during therapeutic manipulations.



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **2 Testing Principle**

p53 autoantibodies are detected by an ELISA assay. Highly purified recombinant wild type human p53 protein is bound to microtiter plates. A peroxidase conjugated goat anti-human antibody catalyses tetramethylbenzidin oxidation resulting in a colorimetric reaction.

## **3 Warnings and recommendations**

This ELISA kit is for in vitro use only. Do not mix reagents from different kits. Calibrator, Negative Control and Positive Control have been tested and shown to be negative for antibodies against HIV (1+2) Hepatitis B and Hepatitis C antigens. However, since there is no absolute guarantee for absence of an infectious agent, these serum controls must be treated as potentially infectious and handled with care.

## **4 Equipment not provided**

- micropipets for 0,5 - 10µl and 50 - 200µl volume
- multipipet or 8-channel pipet for 50µl-, 100µl- and 200µl volume
- automatic plate washer (manual washing possible)
- microtiter plate reader (450 nm filter, reference wavelength >620 nm)

## **5 Reagents provided**

[classification in terms of health hazards / risk phrases / safety phrases]

All unopened reagents are stable until expiration date if stored at 2-8 °C .

### **Microtiter plate**

Twelve strips of eight wells coated with highly purified human recombinant p53 protein.

### **Clip**

Clip for closing the tin foil.

### **Calibrator**

1,5 ml diluted human serum with defined p53 autoantibody concentration.

### **Negative Control**

Ready to use solution of 1 ml diluted human serum without any p53 autoantibody.

### **Sample dilution buffer (6 vials)**

Freeze dried proteinmatrix with 0,05% sodium azide. Dissolve proteinmatrix in 12 ml distilled H<sub>2</sub>O per vial. The reconstituted buffer is used to dilute samples. [R:28-32; S: 28-45].

### **Detector Antibody**

Ready to use antibody conjugate solution (12 ml), anti-human-IgG peroxidase-conjugate.

### **Positive Control**

0,5 ml diluted human serum with defined p53 autoantibody concentration.



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

**Substrate solution**

Ready to use prestained TMB solution (12 ml) for detecting peroxidase activity (light sensitive).

[**Xn** harmful, contains TMB [R: 23/25-36/37/38;  
S: 24-45].

**Stop solution**

Contains 2 M HCl (7.5 ml; ready to use).

[**C** corrosive, contains hydrochloride R: 34-36-37; S: 26-45].

**Wash buffer**

Concentrated buffer (20x). Dilute 50 ml concentrate in 950 ml distilled water.

**5.1 Risk and safety statements**

<b>R 10:</b>	inflammable
<b>R 28:</b>	toxic if swallowed
<b>R 32:</b>	production of toxic gas after contact with acid
<b>R 34:</b>	causes burns
<b>R 36:</b>	irritating to eyes
<b>R 37:</b>	irritating to respiratory system
<b>R 23/25:</b>	toxic after breathing and swallowing
<b>R 36/37/38:</b>	irritating to eyes, respiratory system and skin
<b>S 7:</b>	keep container tightly closed
<b>S 16:</b>	keep away from pilot light; do not smoke
<b>S 24:</b>	avoid contact with skin
<b>S 26:</b>	in case of contact with eyes, rinse immediately with plenty of water and seek medical attention
<b>S 45:</b>	in case of illness, seek immediate medical attention



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **6 Storage and expiry**

<b>reagent</b>	<b>storage</b>		<b>expiry</b>
<b>Microtiter plate</b>	opened/closed	2-8 °C	until expiration date
<b>Calibrator</b>	opened	2-8 °C	until expiration date
<b>Negative Control</b>	opened	2-8 °C	until expiration date
<b>Positive Control</b>	opened	2-8 °C	until expiration date
<b>sample dilution buffer</b>	freeze dried	2-8 °C	14 days
	reconstituted	2-8 °C	
<b>detector antibody</b>	opened	2-8 °C	until expiration date
<b>Substrate solution</b>	opened	2-8 °C / dark	
<b>Stop solution</b>	opened	2-8 °C or RT	
<b>Wash buffer</b>	opened/concentrated	2-8°C or RT	
	opened/diluted	2-8 °C	

### **6.1 Waste disposal**

Remaining chemicals and mixed reagents should be treated as special waste according to official instructions

## **7 Sample collection and preparation**

Collect the blood in a tube without any additives. After coagulation at room temperature, separate serum from the clot by centrifugation.

Samples may be stored for 24 hours at 2 - 8°C. If the samples cannot be tested within 24 hours, they should be aliquoted and stored at -20°C. Avoid repeated freezing and thawing.

The samples must be diluted 1:100 in reconstituted sample dilution buffer before assaying (e.g. 3 µl serum + 297 µl buffer).

For determination of p53 autoantibody titer see 10 (interpretation of results).



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **8 Assay Procedure**

### **8.1 Reagent preparation**

- Allow all reagents reach room temperature before use.
- Fix required strips to the microtiter plate. Unused strips should be stored in the tin foil closed with the clip provided.
- Prepare **wash solution** by diluting the wash buffer (50 ml + 950 ml distilled water).
- Reconstitute freeze dried sample dilution buffer with 12 ml distilled water.
- The samples must be diluted 1:100 in reconstituted sample dilution buffer before assaying (e.g. 5 µl serum + 495 µl buffer). Use diluted calibrator, 1:3 diluted Positive Control and undiluted Negative Control (100 µl/well). We recommend assays in duplicate.

### **8.2 Working procedure**

1. Secure the required strips to the plate. Add 200 µl diluted wash buffer to each well (use multipipet or 8 - channel pipet). Wait for 3 minutes invert plate over sink and shake the plate vigorously. Drain residual fluid by tapping plate on filter paper.
2. Add 100 µl per well of blank (zero control, sample dilution buffer without antibody), controls (diluted calibrator, undiluted Negative Control, 1:3 diluted Positive Control) and diluted samples in duplicates. Incubate 1 hour at room temperature. Wash all wells for a total of five washes using diluted wash buffer.

Washing may be done manually as follows: Add 200 µl diluted wash buffer to each well. Leave the wash buffer in each well for a minimum of 10 seconds per washing cycle, then empty the wells. Invert plate over sink and shake the plate vigorously. Drain residual fluid by tapping plate on filter paper. Repeat washing for a total of five washes.

3. Add 100 µl **detector antibody solution** (ready to use) to each well (use multipipet or 8-channel pipet). Incubate for 1 hour at room temperature. Wash microtiter plate as described in step 2.
4. Immediately after washing in step 3, add 100 µl of **substrate solution** (ready to use) to each well (use multipipet or 8-channel pipet). Incubate for 30 minutes at room temperature in the dark (e.g. in cupboard).
5. Add 50 µl of **stop solution** to each well in order to stop the enzymatic reaction (use multipipet or 8-channel pipet); this will lead to a change of colour. (**Note:** To avoid assay drift it is important to add the stop solution at the same time intervals as the substrate solution.)
6. Before measuring, make sure that the bottom of the microtiter plate is clean.  
Read absorption at 450 nm. We recommend using a reference filter (of 630 nm or 750 nm) in order to compensate for possible differences in the material of the microtiter plate (Instead of using a reference filter it is possible to subtract the  $E_{450}$  of Wash buffer from all  $E_{450}$  values.)





**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **9 Analysis**

Read absorption at 450 nm (reference wavelength 620nm or 750nm recommended). (Instead of using a reference filter it is possible to subtract the  $E_{450}$  of Wash buffer from all  $E_{450}$  values.)

### **9.1 Allowed absorption range of controls**

After correct carrying out, the absorption of the controls should be in the range as detailed below.

	Calibrator (1:2 diluted)	Negative Control
$E_{450-630}$	$> 0.5$	$\leq 0.3$

### **9.2 Internal Quality Assurance**

Part of the kit is a positive control for enabling an internal Quality Assurance. The Positive Control contains p53 autoantibodies in a concentration of 14 U/ml (1,4 U/test). As internal control a 1:3 dilution of the Positive Control should be included in each measurement. For the assay run to be valid calculation of Positive Control titer should result in 1,4 U/test ( $\pm 25\%$ )

## **10 Interpretation of results**

Construct a calibration curve using different dilutions of calibrator and zero control. This calibration curve is a linear regression curve which cuts the x-axis at 0. Cut-off is 120 U/ml, samples should be defined as negative if their titer is  $\leq 120$  U/ml.

**negative samples:** Titer  $< 120$  U/ml

**positive samples:** Titer  $> 120$  U/ml

**critical:** Titer 60-120 U/ml

Serum samples having a titer of 60-120 U/ml are defined “critical” meaning that there is a probable presence of very small quantity p53 autoantibodies. Patients should be controlled 5-8 weeks later again.

**1 Unit is defined as p53 binding activity which corresponds to the binding activity of 100  $\mu$ l undiluted calibrator.**



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## 10.1 Example

### Dilution of calibrator

Dilute the calibrator (10U/ml) with sample dilution buffer. Use undiluted, diluted calibrator and zero control for calculating the calibration curve. We recommend 4-6 measurements and an additional zero control. The absorption ( $E_{450}$ ) of undiluted calibrator corresponds to exact 1 Unit (1U/test).

dilution	undiluted	1:2	1:4	1:8	1:16	Zero control
titer [U/test]	1	0,50	0,25	0,125	0,063	0,0
sample dilution buffer (µl)	-	100	150	175	187,5	100
calibrator (µl)	100	100	50	25	12,5	0,0

### Example for titer calculation

	Dilution	$E_{450-620}$	U/test <sup>Δ</sup>	U/test <sub>undiluted</sub>	U/ml
zero control		0,004	0,00		
calibrator	1:16	0,139	0,063		
calibrator	1:8	0,278	0,125		
calibrator	1:4	0,548	0,25		
calibrator	1:2	1,045	0,5		
calibrator	undiluted	2,319	1,00		
sample 1	1:100	0,2	0,10	10	100
sample 2	1:100	0,48	0,22	22	220
sample 3	1:100	0,99	0,44	44	440
sample 4	1:100	2,62	*		
sample 4	1:1000	0,88	0,39	390	3900
Negative Control <sup>&amp;</sup>	undiluted	0,129	0,07	7	70
Positive Control	1:3	1,054	0,47	1,41	14,1

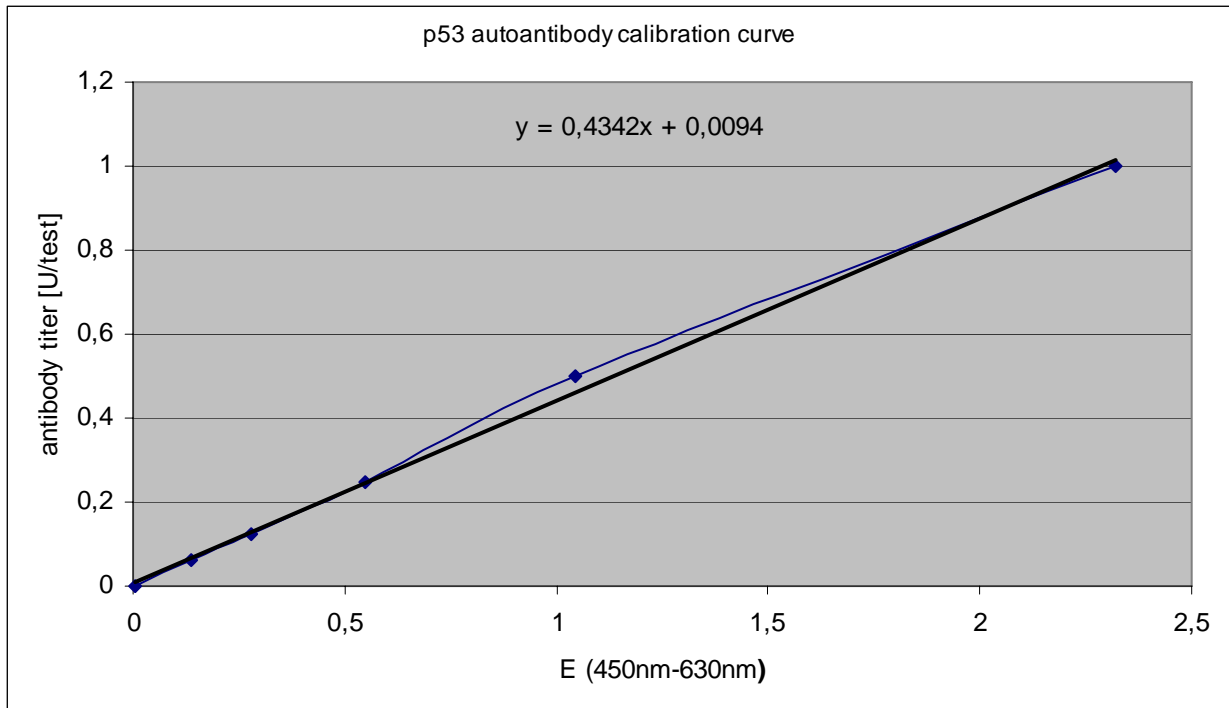
\* out of calibration range

<sup>Δ</sup> test volume 100 µl

& Negative Control in the kit is diluted 1:100 (ready to use)



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**



(do not use this calibration curve for your calculations)

### Serum samples

Dilute your positive serum samples in sample dilution buffer that the absorption is in the range of your calibration curve. It is possible to dilute until 1:5000. Absorptions of samples which are out of calibration range must be tested again in higher dilution. On calculation of antibody titer of serum consider the correspondent dilution.

$\text{antibody titer}_{\text{calibration curve}} [\text{U/test}] \times \text{dilution}_{\text{serum}} = \text{antibody titer}_{\text{serum undiluted}} [\text{U/test}]$

$\text{antibody titer}_{\text{serum undiluted}} [\text{U/test}] \times 10 = \text{antibody titer}_{\text{serum undiluted}} [\text{U/ml}]$

### Positive Control / Negative Control

This kit contains prediluted serum with a defined concentration of p53 autoantibodies (14 U/ml) as a positive control. The Negative Control consists of a 1:100 dilution of serum containing no p53 autoantibodies.



STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE

## 11 Trouble shooting

### High background ( $E_{\text{calibrator, 1:2 diluted}} > 2,0$ )

Possible reason for high background is wrong washing. Stringent washing is recommended. If you use an automatic microtiter plate washer, check pressure and time period of washing. In case of manual washing be sure to drain residual fluid almost completely after every 5 washing steps.

### Low absorption of calibrator ( $E_{\text{calibrator, 1:2 diluted}} < 0,5$ )

Possible reason for low absorption of calibrator is too strong pressure during washing. In case of using an automatic microtiter plate washer reduce washing pressure or washing cycles.

### $E_{\text{negative control}} \geq 0,30$

If the absorption of Negative Control is too high, this could mean that incubation times of calibrator and Negative Control were different. By measuring lots of samples simultaneously avoid large delay during pipetting. For registration of possible delays we recommend pipetting of controls (calibrator and Negative Control) in the first and last wells of microtiter plate.



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

## **12 Test-Characteristics**

### **Specificity**

The p53 autoantibody ELISA detects IgG antibodies specific for p53 protein. Cross reactivity of other antibodies were not observed. The detection limit is 0,07 U/test.

### **Linearity**

The linearity of the assay was established using 5 dilutions of a positive serum in duplicates. The p53 autoantibody ELISA shows linearity in a range from 0-1 U/assay.

### **Reproducibility**

Reproducibility of intra- and inter-assay variability was demonstrated using a positive control.

Intra-assay-variation:

Intra-assay-variation was measured using 15 test points. The intra-assay-variation coefficient was  $< 10\%$ .

Inter-assay-variation:

Inter-assay-variation was obtained using two kits. The inter-assay-variation coefficient was shown to be  $< 15\%$ .

### **Cut-off**

The Cut-off was defined as twice the arithmetic mean absorption value of 189 healthy blood donors. The cut off defines the threshold limit of autoantibody absence. Samples should be defined as negative if their titer is  $\leq 120$  U/ml



**STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE**

### **13 Literature**

1. M. LECHPAMMER, J. LUKAC S. LECHPAMMER D. KOVACEVIC M. LODA, Z. KUSIC  
*Humoral immune response to p53 correlates with clinical course in colorectal cancer patients during adjuvant chemotherapy*  
Int J Colorectal Dis **19**: 114–120 (2004)
2. M. SHAARAWY AND M. SHEIBA  
*Diagnostic and Prognostic Significance of Circulating Tumor Suppressor Gene p53 Autoantibodies in Patients with Gestational Trophoblastic Tumors*  
Acta Oncologica, **43**(1):43-48 (2004)
3. JIAN-YING ZHANG ET AL.  
*Enhancement of Antibody Detection in Cancer Using Panel of Recombinant Tumor-associated Antigens*  
Cancer Epidemiology, Biomarkers & Prevention, **12**: 136-143 (2003)
4. GADDUCCI, A., FERDEGHINI, M., BUTTITA, F., FANUCCHI, A., ANNICCHIARICO, C., PRONTERA, C., BEVILACQUA, G., GENAZZANI, A.R.  
*Preoperative serum antibodies against the p53 protein in patients with ovarian and endometrial cancer*  
Anticancer Research **16**, 3519-3524 (1996)
5. ANGELOPOULOU, K., DIAMANDIS, E.P., SUTHERLAND, D.J.A., KELLEN, J.A., BUNTING, P.S.  
*Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers*  
Int. J. Cancer **58**, 480-487 (1994)
6. SHIN, D.M., KIM, J., RO, J.Y., HITTELMAN, J., ROTH, J.A., HONG, W.K., HITTELMAN, W.N.  
*Activation of p53 gene expression in premalignant lesions during head and neck tumorigenesis.*  
Cancer Res. **54**, 321-326 (1994)
7. HOUBIERS J.G.A., VAN DER BURG S.H., VAN DE WATERING L.M.G., TOLLENAR R.A.E.M., BRAND A., VAN DE VELDE C.J.H., MELIEF C.J.M.  
*Antibodies against p53 are associated with poor prognosis of colorectal cancer*  
Br J Cancer **72**: 637-641 (1995)
8. ANGELOPOULOU K, STRATIS M, DIAMANDIS EP  
*Humoral immune response against p53 protein in patients with colorectal carcinoma*  
Int. J. Cancer **70**: 46-51 (1997)
9. ANGELOPOULOU K, ROSEN B, STRATIS M, YU H, SOLOMOU M, DIAMANDIS EP  
*Circulating antibodies against p53 protein in patients with ovarian carcinoma. Correlation with clinopathologic features and survival*  
Cancer **78**: 2146-2152 (1996)
10. VOGL FD, FREY M, KREIENBERG R, RUNNEBAUM IB  
*Autoimmunity against p53 predicts invasive cancer with poor survival in patients with an ovarian mass*  
British J. Cancer, **83** 1338-1343 (2000)
11. ZALCMAN G, SCHLICHTHOLZ B, TREDANIEL J, URBAN T, LUBIN R, DUBOIS I ET AL  
*Monitoring of p53 autoantibodies in lung cancer during therapy: relationship to response to treatment.*  
Clin. Cancer Res., **4**: 1359-1366 (1998)



STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE

12. TAKEDA A, SHIMADA H, NAKAJIMA K, IMASEKI H, SUZUKI T, ASANO T, OCHIAI T, ISONO  
*Monitoring of p53 autoantibodies after resection of colorectal cancer: relationship to operative curability.*  
Eur J Surg., **167**: 50-53 (2001)
13. CASTELLI M, CIANFRIGLIA F, MANIERI A, PALMA L, PEZZUTO R, FALASCA G, DELPINO A  
*Anti p53 and anti heat shock proteins antibodies in patients with malignant or pre-malignant lesions of the oral cavity.*  
Anticancer Res, **21**: 753-758 (2001)
14. SITRUK V, VAYSSE J, CHEVRET S, GANNE-CARRIE N, CHRISTIDIS C, TRINCHET J, BEAUGRAND M  
*Prevalence and prognostic value of serum anti-p53 antibodies in hepatocellular carcinoma. A study of 159 patients.*  
Gastroenterol Clin Biol, **24**: 1159-1163 (2000)
15. ROHAYEM J, CONRAD K, ZIMMERMANN T, FRANK K-H  
*Comparison of the Diagnostic Accuracy of Three Commercially Available Enzyme Immunoassays for Anti-p53 Antibodies*  
Clin Chem, **45**: 2014-2016 (1999)
16. FLAMMANN HT, KUHN H-M,  
*p53 Autoantibodies and Cancer: Specificity, Diagnosis and Monitoring.*  
IN: CANCER AND AUTOIMMUNITY (EDS. SHOENFELD Y, GERSHWIN ME) ELSEVIER, AMSTERDAM. PP. 181-191 (2000)
17. KUHN H, KROMMINGA A, FLAMMANN H, FREY M, LAYER P, ARNDT R  
*p53 autoantibodies in patients with autoimmune diseases: a quantitative approach*  
Autoimmunity, **31**: 229-235 (1999)



STEINBEIS-TRANSFERZENTRUM  
FÜR ANGEWANDTE BIOLOGISCHE CHEMIE

## 14 Assay summary

### Procedure

- Prepare wash solution  
50 ml concentrate + 950 ml distilled water
- Reconstitute freeze dried **sample dilution buffer** with  
12 ml distilled water.
- dilute serum samples 1:100 in reconstituted **sample dilution buffer**.
- Prepare strips of microtiter plate (For hydration add 200 µl  
diluted wash buffer to each well and wait for 3 minutes).
- Pipet 100 µl per well of **controls (diluted calibrator, Negative Control,  
diluted Positive Control) or diluted samples** in duplicate.
- Wash microtiter plate strips with diluted **wash solution**  
(5 x automatically or 5 x manually).
- Pipet 100 µl per well of **detector antibody**
- Wash
- Pipet 100 µl per well of **substrate solution**.
- Pipet 50 µl per well of **stop solution**.
- Measure at 450 nm  
(recommended reference filter: 620 or 750 nm).
- Time required for the assay: approximately 3 hours.

### Incubation

1 hr; RT

1 hr; RT

30 min; RT;  
**in the dark !**