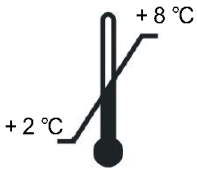
Manual

GST-π ELISA Kit

For the in vitro determination of Glutathion-S-Transferase π in Serum, Plasma and tumor tissue extract

Gültig ab / valid from 04.09.2006

K 7960



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1. INTENDED USE

The *Immundiagnostik* Assay is intended for the quantitative determination of **GST-π** in serum and tumor tissue extract. For in vitro diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Glutathion-S-transferases (GST) are a group of enzymes, which are involved in detoxification processes. The GSTs are divided in 5 subgroups: Alpha, Mu, Pi, Teta and microsomal. The classes differ in their physicochemical, immunological, enzymatic and structural properties. Their function in cellular metabolism is the coupling of electrophile compounds, for example most of the carcinogens, to Glutathion (GSH). The GST Pi is the main group in erythrocytes and in some leucocytes, moreover they are found in most tissues except liver. The GST Pi are dimeric molecules with a molecular weight of 46000 Da and an isoelectric point of about 4,7. High GST Pi levels were found in all neoplastic tissue (fetal, maligne) and in serum of patients with post-operative relapse. Increased serum levels were also found in haematological diseases (haemolytic anaemia and sub-groups of leukemia). Moreover GST Pi concentrations give information about the multi-drug-resistance-status of tumours.

3. PRINCIPLE OF THE TEST

This Enzyme-Immuno Assay (EIA) allows the quantitative determination of human GST Pi. GST Pi is first coated to the surface of the microtiter plates. After a blocking step and a preincubation of calibrators and samples with a polyclonal rabbit antibody the GST-pi in the controls and samples compete with the GST-pi on the plate for the antibody binding. After a washing step the detection of the bound rabbit antibody is performed by a peroxidase labeled goat anti rabbit antibody (POD-antibody). The amount of converted substrate (TMB) is indirectly proportional to the amount of GST-Pi antigen in the sample and can be determined photometrically at 450 nm.

4. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
K 7960MTP	PLATE	One holder with precoated strips	96 wells
K 7960WP	WASHBUF	ELISA wash buffer concentrate 10x	100 ml
K 7960AP	ASSAYBUF	Assay buffer, ready-to-use	11 ml
K 7960A1	1.AB	1st Antibody (rabbit anti hGST-pi), lyophilized	12 ml
K 7960K	CONJ	POD antibody, (goat anti rabbit, Peroxidase-labeled), ready-to-use	11 ml
K 7960ST	STD	Calibrators, lyophilized (0; 9.4; 37.5; 150; 600 ng/ml)	2 x 5 vials
K 7960KO	CTRL	Control, lyophilized	2 vials
K 7960TMB	SUB	TMB substrate (Tetramethylbenzidine)	1 x 15 ml
K 7960AC	STOP	ELISA stop solution, ready-to-use	1 x 7 ml

On demand we will send you 1 calibrator set free of charge. Any further calibrator sets will be charged.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized water
- Precision pipettors calibrated to deliver 50-100 µl
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Microplate reader 450 nm

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- The ELISA **WASHBUF** (wash buffer concentrate) must be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37 °C in water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- Lyophilized **STD** (standards) and **CTRL** (control) are stable at 2 -8 °C until expiry date stated on the label. Lyophilized **STD** (standards) and **CTRL** (control) must be reconstituted with **100 µl** aqua bidest. Allow the vial to stand for minimum 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards and control can be frozen and thawed once.

- The lyophilized **1.AB** (1. antibody, rabbit anti-GST π) must be stored at **2-8 °C**. The lyophilized **1.AB** must be reconstituted with **12 ml** aqua bidest. Allow the vial to stand for minimum 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. The reconstituted 1. antibody must be stored at 20°C. The reconstituted 1. antibody can be frozen and thawed up to 4 times and is stable at 20°C until the expiry date stated on the label.

- All other test reagents are ready to use. Test reagents are stable until the expiry date stated on the label of test package when stored at **2-8 °C**.

7. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Quality control guidelines should be observed.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

8. SPECIMEN COLLECTION AND PREPARATION

Plasma or **serum:** Collect blood in plasma- or serum tubes (don t use heparin tubes), mix and incubate 15-30 minutes at 2-8°C, then centrifuge 10 minutes at 1800xg. Store serum or plasma at -20°C.

Caution - Don t use haemolytic serum or plasma -

Tissue material has to be homogenized (e.g. mechanically with a dismembrator) and then resuspended in phosphate buffer. Then follows an ultra centrifugation step (1h at 100.000 x g). The supernatant of this centrifugation step is the cytosolic fraction. Protein in the supernatant should be determined according to Lowry et al. or with BCA Protein Assay [Pierce].

Alternatively the tissue material can be resuspended after the homogenization in phosphate buffer with 1% Triton X100. After mixing for at least 2 hours this tissue lysate has to be centrifuged at 10 000 x g for 10 minutes. After determination of protein content the supernatant can be used for the EIA

9. ASSAY PROCEDURE

Procedural notes

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

Test procedure

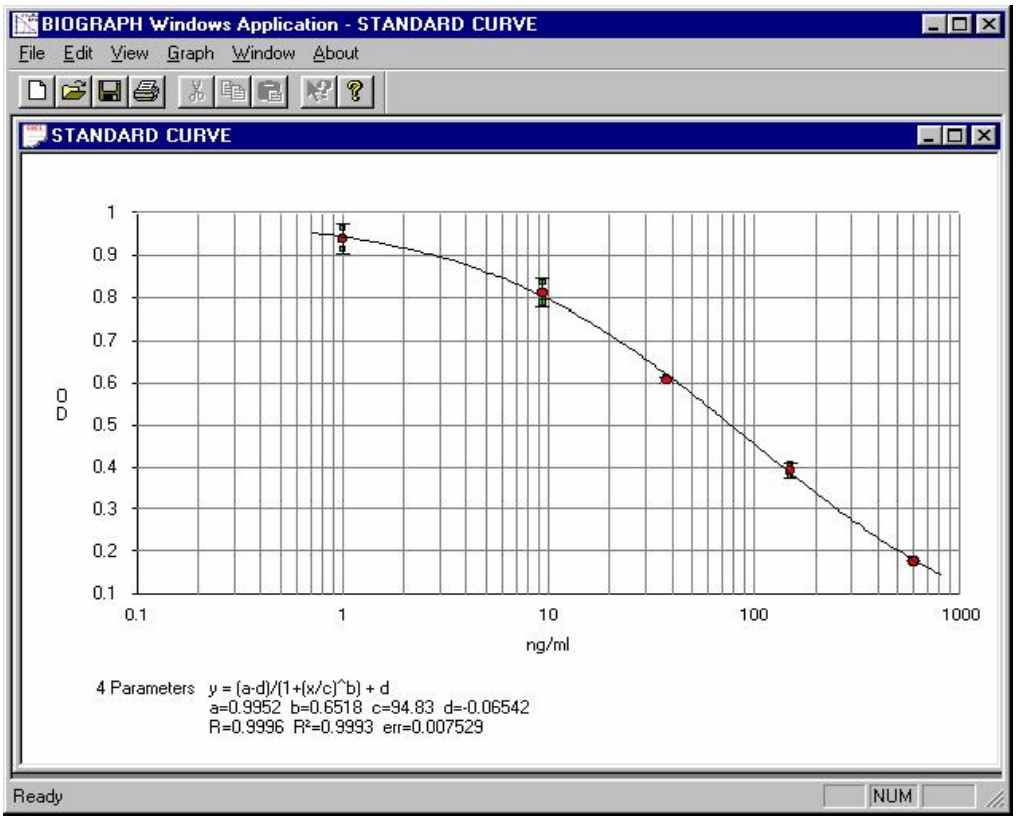
Preincubation of standard/control/ sample with antibody:

1. Pipette **25 µl STD** (standards), **CTRL** (control) and patient samples and **25 µl ASSAYBUF** (Assay buffer) into test tubes.
2. Add **200 µl** of the **1.AB** (1st antibody).
3. Incubate for **16h** at **2 - 8°C**
4. Wash the precoated microtiter plate 5 x with 250 µl ELISA wash buffer. Carry out the tests in duplicate.
5. Shake the preincubated solution well and pipette **100 µl** of the solution in each well of washed microtiter plate.
6. Incubate for **4h** at room temperature °C, shaking on a horizontal mixer.
7. Decant the content of plate and wash the wells **5 x with 250 µl** ELISA wash buffer
8. Pipette **100 µl CONJ** (peroxidase labeled antibody) into each well.
9. Incubate for **45 min** at room temperature, shaking on a horizontal mixer.
10. Decant content of plate and wash the wells **5 x with 250 µl** ELISA wash buffer
11. Pipette **100 µl SUB** (TMB substrate solution) into each well
12. Incubate **10-20 minutes** at room temperature until sufficient colour differences are visible.
13. Add **50 µl STOP** (stop solution) and mix shortly.
14. Determine absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

10. RESULTS

A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.
THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL algorhithm is recommended.

Typical calibration curve



Concentration [ng/ml]	600	150	37.5	9.4	0
B/B0 [%]	15	36	68	89	100

The data is for demonstration only and cannot be used in place of data generations at the time of the assay.

11. LIMITATIONS

Samples with GST-pi levels greater than the highest calibrator should be diluted and re-assayed.

12. QUALITY CONTROL

Immundiagnostik recommends commercial control samples for internal quality control.

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

Expected values

Plasma / Serum

90 280 ng/ml

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) of the Immundiagnostik GST-pi ELISA test was calculated from 10 replicate determinations on each of the samples.

Intra-Assay CV n= 10

Sample	GST-pi Mean value [ng/ml]	Intra-Assay CV [%]
1	50	12

The total precision (inter-assay variation) of the Immundiagnostik GST-pi ELISA test was calculated from data on 2 samples obtained in 10 different assays by three technicians on two different lots of reagents over a period of 3 months.

Inter-Assay CV n= 10

Sample	GST-pi Mean value [ng/ml]	Inter-Assay CV [%]
1	84	15

14. LITERATURE

1. Sundberg et al. *Nephron* 1994; 66:162-169
2. Hayes, Pickett, Mantle, *Proceedings of 3rd International GST Conference*, Edinburgh, Scotland 1989
3. Sundberg et al. *Nephron* 1994; 67:308-316

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG or Apotech Corporation along with a written complaint.