Glutathione S-Transferase (GST) Assay Kit

For the determination of GST activity in biological samples

Valid from 31.01.2013
1. INTENDED USE

The Glutathione S-Transferase (GST) Assay Kit is intended for the measurement of total GST activity. It can be used to measure GST activity in cell and bacterial lysates, tissue homogenates, and in erythrocyte lysates. It is for research use only.

2. INTRODUCTION

Glutathione-S-transferases (GSTs) are a group of enzymes that are important in the detoxication of many different xenobiotics in mammals. The enzymes protect cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics, and thereby defend cells against the mutagenic, carcinogenic, and toxic effects of the compounds. GST activity was found to be present in plants, insects, yeast, bacteria, and in most mammalian tissues, especially in the liver, which plays a key role in detoxification. There are several classes of GST isoenzymes that differ in their specificity toward xenobiotic or endogenous substrates.

The Glutathione S-Transferase (GST) Assay Kit utilizes 1-Chloro-2,4-dinitrobenzene (CDNB) which is suitable for the broadest range of GST isoenzymes. Upon conjugation of the thiol group of glutathione to the CDNB substrate, there is an increase in the absorbance at 340 nm.

Indication
- Detoxification marker

3. PRINCIPLE OF THE TEST

GST catalyzes the conjugation of L-glutathione to CDNB through the thiol group of the glutathione.

\[
\text{GST} \\
\text{GSH} + \text{CDNB} \rightarrow \text{GS-DNB Conjugate} + \text{HCl}
\]

The formation of the GS-DNB conjugate is proportional to the enzyme activity and can be used for photometric GST activity determination. The rate of increase in the absorption at 340 is directly proportional to the GST activity in the sample.

One unit of GST activity is defined as the amount of enzyme producing 1 mmol of GS-DNB conjugate per minute under the conditions of the assay.
Arbeitsanleitung/Manual

GST – Aktivität/Activity

4. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 2631AP</td>
<td>ASYBUF</td>
<td>GST Assay buffer</td>
<td>2 x 65 ml</td>
</tr>
<tr>
<td>K 2631KO</td>
<td>CTRL</td>
<td>GST Positive control</td>
<td>10 x 1 vial</td>
</tr>
<tr>
<td>K 2631SOLA</td>
<td>SOL A</td>
<td>Substrate (CDNB)</td>
<td>10 x 120 μl</td>
</tr>
<tr>
<td>K 2631SOLB</td>
<td>SOL B</td>
<td>L-Glutathione reduced</td>
<td>10 x 120 μl</td>
</tr>
<tr>
<td>K 2631PV</td>
<td>SAMPLEBUF</td>
<td>Sample dilution buffer</td>
<td>1 x 80 ml</td>
</tr>
</tbody>
</table>

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Temperature controlled UV/visible spectrophotometer
- 1 ml disposable cuvette or Quartz cuvette
- Precision pipettors and disposable tips to deliver 5-1000 μl
- A multi-channel dispenser or repeating dispenser
- Bidistilled water
- Standard laboratory glass or plastic vials, 1,5 ml
- Centrifuge capable of 14000 x g
- Vortex-Mixer

6. SAMPLE PREPARATION

Erythrocytes

Add 200 μl SAMPLEBUF (Sample dilution buffer) to 50 μl of frozen EDTA whole blood sample or CTRL POS (positive control), vortex for 10 sec.

Centrifuge at 14,000 x g for 10 minutes, if possible under cooling.

Collect supernatant and use for the assay.

The deep freezing of the whole blood sample und its subsequent treatment with the SAMPLEBUF (sample dilution buffer) ensures the complete lysis of the erythrocytes.
7. PREPARATION AND STORAGE OF REAGENTS

- **SOL A** (Substrate), **CTRL** (control) and **SOL B** (glutathione) are stable at -20 °C until the expiry date stated on the label.
- Thaw **ASYBUF** (GST assay buffer) and **SAMPLEBUF** (sample dilution buffer) and bring to room temperature before use in the assay.
- Thaw **SOL A** (substrate) and **SOL B** (glutathione) and bring to room temperature immediately before use in the assay.
- **Preparation of reaction mixture for 10 determinations:**
  
<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASYBUF</td>
<td>9,8 ml</td>
</tr>
<tr>
<td>SOL A</td>
<td>0,1 ml</td>
</tr>
<tr>
<td>SOL B</td>
<td>0,1 ml</td>
</tr>
</tbody>
</table>

  The addition of SOL A may cause a light turbidity, which disappears upon carefully mixing the solution.

  **Note:** the substrate solution must be prepared fresh before each assay series. It can be stored for maximal 60 minutes in a light protected vial.

8. ASSAY PROCEDURE

**Test procedure**

Set the spectrophotometer at 340 nm. On a kinetic program: read every 30 seconds over a period of 5 minutes after a lag time of 1 minute.

**Blank measurement:** Transfer 1000 μl of the reaction mixture solution to a cuvette, wait until a temperature of 25 °C is achieved and maintain at that temperature. Read the increase in the absorption \([\Delta A_{340}/\text{min}]\) over a period of 5 minutes after a lag time of 1 minute.

**Sample measurement:** Add 955 μl reaction mixture solution directly to the cuvette, wait until a temperature of 25 °C is achieved and maintain at that temperature. Add 5 μl of the pre-treated sample and mix well. Read the increase in the absorption \([\Delta A_{340}/\text{min}]\) over a period of 5 minutes after a lag time of 1 minute.

Pay attention, that the increase in the absorption \([\Delta A_{340}/\text{min}]\) is linear. In all other cases, the sample must be diluted in **SAMPLEBUF** (sample dilution buffer) and re-assayed.
9. RESULTS

Calculations
The increase in absorbance \([\Delta A_{340}/\text{min}]\) is directly proportional to the GST activity.

Sample and blank
\[ \Delta A_{340\text{nm}/\text{min}} = A_{340\text{nm}} \text{(Stop)} - A_{340\text{nm}} \text{(Start)} \]

Subtract the \(\Delta A_{340\text{nm}/\text{min}}\) of the blank from the \(\Delta A_{340\text{nm}/\text{min}}\) of the sample. Use this rate for the calculation of the GST specific activity.

Equation for the GST specific activity
\[
(\Delta A_{340\text{nm}/\text{min}}) \times V \text{ (ml)} \times \text{dil} \times \frac{1}{\varepsilon_{\text{mM}}} = \frac{\mu \text{mol/ml/min}}{V_{\text{sample}} \text{ (ml)}}
\]

Where:
\(\varepsilon_{\text{mM}} \text{ (mM}^{-1}\text{cm}^{-1})\) = The extinction coefficient for CDNB conjugate at 340 nm
For a cuvette: 9.6 mM\(^{-1}\) (path length 1 cm)
For a cuvette with a different path length, the coefficient should be calculated using this substitution factor:
\(\varepsilon_{\text{mM}} = 9.6 \times \text{path length in cm}\)

\(V = \text{the reaction volume (1 ml)}\)
\(\text{dil} = \text{the dilution factor of the original sample}\)
\(V_{\text{sample}} = \text{the volume of the enzyme sample tested (e.g. 0.005 ml)}\)

10. LIMITATIONS

If the GST control or GST sample is too concentrated, it must be diluted with SAMPLEBUF prior to the assay. The dilution factor must be considered.
11. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

12. PRECAUTIONS

- For research 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.

13. TECHNICAL HINTS

- 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 colorless until use.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.
14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the kit package are for research 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 along with a written complaint.

15. REFERENCES

Used symbols:

- **Temperature limitation**
- **RUO** For research use only
- **Manufacturer**
- **LOT** Lot number
- **REF** Catalogue Number
- **Contains sufficient for <n> tests**
- **Use by**