جامعة الملك سعود
King Saud University
1957

## BCH 447 Practical Metabolism

# Estimation of Glutathione in Plasma

# Objectives

To estimate the amount of glutathione in serum sample using a standard curve

# Glutathione

- It occurs naturally as a **tripeptide** (gamma-**glutamyl*cysteinyl*glycine** )
- It has **two form**: reduced form (**GSH**) and oxidized form (**GSSG**)
- It has reducing and nucleophilic properties, those properties due to the presence of **Sulfhydryl group or thiol group (-SH).**

- **Reducing**, means it will give the other protein the H ion to maintain them in reducing form.
- **Nucleophilic,** means GSH will loss the H ion then become nucleophilic (contain negative charge) can react with the compound which carrying positive charge.

# Functions of Glutathione

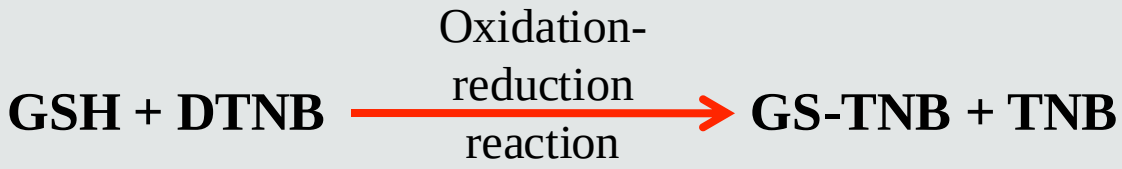
- It acts as anti-oxidant, blood booster, and cell detoxifier in the body.

| **GSH** is crucial to a variety of life processes, including | | | | |
|---|---|---|---|---|
| **Amino acid transport across membrane** | **Maintenance of the SH level of proteins** | **Detoxification of xenobiotics such as drugs** | **Removal of hydroperoxide** and **free radicals**. | **Coenzyme with glutathione peroxidase, glutathione S-transferase** and **thiol transferase.** |

- Physiological values of intracellular GSH generally range **from 1 to 10 mM.**
- Glutathione deficiencies have been linked to many forms of **cancer**.

# Assay Principle

- The principle of the assay is based on the **oxidation of the reduced form of glutathione (GSH)** by the aromatic disulphide compound [5,5-dithiobis-2-nitrobenzoic acid (DTNB)] to form GS-TNB and the aromatic thiol, 5-thio-2-nitrobenzoic acid (TNB).




- **Yellow color is formed (TNB)** It can be measured **at 412nm** and is proportional to the amount of glutathione present in the sample.

**Note:** DTNB is irritating to the eyes, respiratory system and skin. Wear suitable protective clothing.

**Note** : This method is also useful to determine the GSSG.

First , GSSG will be converted to GSH by glutathione reductase (GR) and NADPH assay then it will react with DTNB reagent

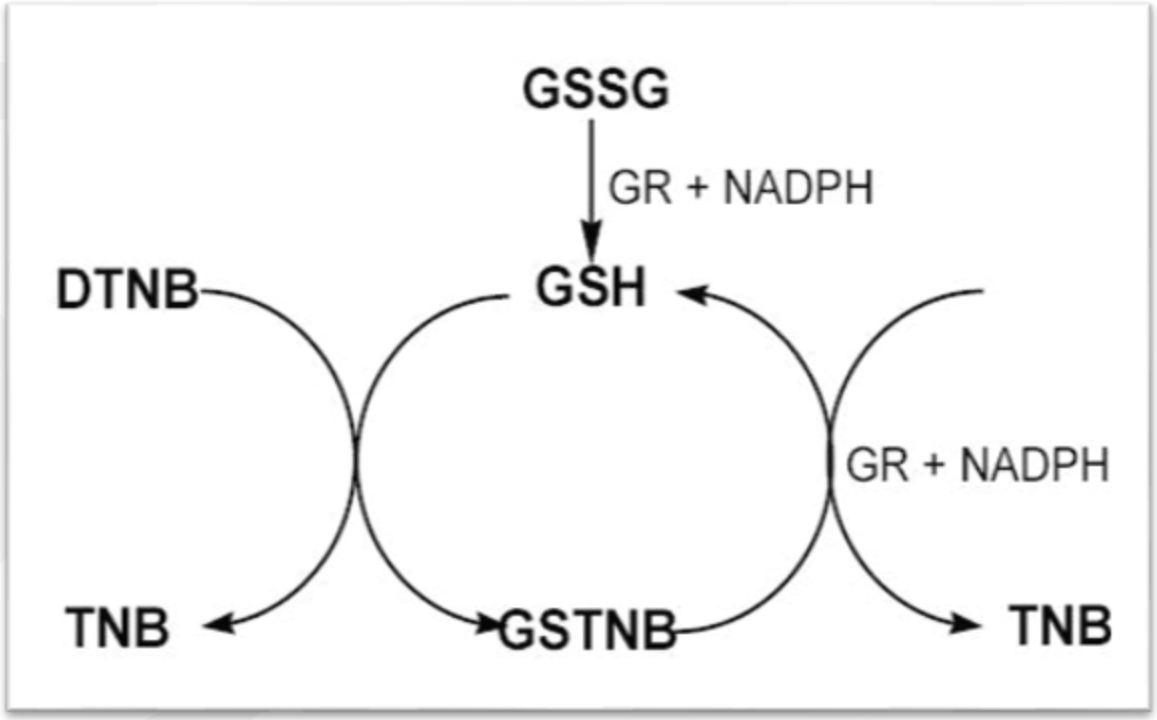



**Figure 3.** Glutathione (GSH) reaction with DTNB reagent

# Method

**1- Prepare serial GSH concentration, as the following table:**

| Tube No. | GSH stock Solution (ml) | Phosphate Solution (ml) | Total Volume (ml) | GSH Conc. (mg/dl) | GSH Conc. (µg/ml) |
|---|---|---|---|---|---|
| **Blank** | 0 | 3 | 3 | 0 | 0 |
| **1** | 0.6 | 2.4 | 3 | 2 | 20 |
| **2** | 1.2 | 1.8 | 3 | 4 | 40 |
| **3** | 1.8 | 1.2 | 3 | 6 | 60 |
| **4** | 2.4 | 0.6 | 3 | 8 | 80 |
| **5** | 3 | 0 | 3 | 10 | 100 |

**2- For standard curve (Tubes from blank to 5)**

**In a separate test tube**, take 0.5 ml of solution (from step 1) + 2 ml of Phosphate Solution + 0.25 ml DTNB + 0.25 ml $H_2O$.

***3- For Sample:*** Take 0.3 ml of sample +2 ml of Phosphate Solution + 0.25 ml DTNB+ 0.45 ml $H_2O$.

**Incubate all tubes for 10min at 37°C → Read absorbance at 412 nm **

# Results

| Tube No. | GSH Conc (mg/dl) | Absorbance At 412 nm |
|---|---|---|
| **1** | 2 | |
| **2** | 4 | |
| **3** | 6 | |
| **4** | 8 | |
| **5** | 10 | |
| **Blank** | 0 | |
| **Sample** | From Standard Curve | |

- Plot the standard curve and determine glutathione concentration from the graph expressed as µg/ml

# Homework

- What are the **7 mechanisms** of antioxidant?
- What are the **three classes** of antioxidant defense systems in human?